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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

Contents

Cancer Treatment Reports
Volume 71, Number 6
June 1987

EDITORIAL

557 Patient Accrual to Clinical Trials. **M. A. Friedman.** National Cancer Institute, Bethesda, Maryland

ORIGINAL ARTICLES

559 Selection Factors in Clinical Trials: Results From the Community Clinical Oncology Program Physician's Patient Log. **C. P. Hunter, R. W. Frelick, A. R. Feldman, A. R. Bavier, W. H. Dunlap, L. Ford, D. Henson, D. Macfarlane, C. R. Smart, R. Yancik, and J. W. Yates.** National Cancer Institute, Bethesda, Maryland

567 Synergistic Effect of Recombinant Interleukin-2 on the Inhibition of Growth of Adenocarcinoma 755 by Mitomycin. **M. Iigo, M. Sakurai, N. Saijo, and A. Hoshi.** National Cancer Center Research Institute, Tokyo, Japan

571 Influence of Daunorubicin and Cytarabine Sequencing on the Outcome of Therapy in Acute Myelogenous Leukemia: A Randomized Trial. **E. Archimbaud, D. Fiere, D. Treille-Ritouet, P. Adeleine, D. Guyotat, C. Sebban, H. Vuvan, and J.-J. Viala.** Université Claude Bernard, Lyon, France

575 Phase II Trial Evaluating Continuous Infusion of Etoposide, Cisplatin, and Hexamethylmelamine in Extensive-Disease Small Cell Carcinoma of the Lung. **S. C. Remick, A. J. Neville, and J. K. V. Willson.** University of Wisconsin Clinical Cancer Center and William S. Middleton Memorial Veterans Administration Hospital, Madison

581 Demonstration of a Schedule-Dependent Therapeutic Synergism Utilizing the Interacting Drugs Methotrexate and Teniposide in L1210 Leukemia. **G. L. Wampler, W. H. Carter, Jr, E. D. Campbell, and I. D. Goldman.** Medical College of Virginia, Virginia Commonwealth University, Richmond

593 Phase I and Pharmacokinetic Study of Menogaril Administered as a 72-Hour Continuous Iv Infusion. **H. J. Long, G. Powis, A. J. Schutt, and C. G. Moertel.** Mayo Clinic, Rochester, Minnesota

599 Pharmacokinetics of High-Dose Teniposide. **J. J. M. Holthuis, L. G. E. de Vries, P. E. Postmus, W. W. J. van Oort, H. Verleun, A. Hulshoff, D. T. Sleijfer, and N. H. Mulder.** University of Utrecht, The Netherlands

605 Combination Chemotherapy in the Treatment of Adenocarcinoma of Unknown Primary Origin. **N. Walach and Y. Horn.** Assaf Harofeh Medical Center, Zerifin, Israel

609 Cardiotoxicity in Patients Treated With Mitoxantrone: Southwest Oncology Group Phase II Studies. **F. J. Mather, R. M. Simon, G. M. Clark, and D. D. Von Hoff.** Southwest Oncology Group

615 Pilot Trial of Prolonged Continuous-Infusion 5-Fluorouracil and Weekly Cisplatin in Advanced Colorectal Cancer. **J. E. Cantrell, Jr, R. D. Hart, R. F. Taylor, and J. H. Harvey, Jr.** St. Vincent's Hospital, Birmingham, Alabama

619 Selective Enhancement by Menadiol of In Vitro Drug Activity in Human Lymphatic Neoplasms. **Y.-Z. Su, T. E. Duarte, P. L. Dill, and L. M. Weisenthal.** Veterans Administration Medical Center, Long Beach, California, and the University of California, Irvine

627 Phase II Study of Mitoxantrone in Epithelial Ovarian Cancer. **F. Lawton, G. Blackledge, J. Mould, T. Latief, R. Watson, and A. D. Chetiyawardana.** University of Birmingham, England

631 Activity of Bisantrene in Refractory Lymphoma. **P. McLaughlin, F. Cabanillas, F. B. Hagemeister, and W. S. Velasquez.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

BRIEF REPORTS

635 Continuous Sc Infusion of Narcotics Using a Portable Disposable Device in Patients With Advanced Cancer. **E. Bruera, C. Brenneis, M. Michaud, S. Chadwick, and R. N. MacDonald.** Cross Cancer Institute, Edmonton, Alberta, Canada

639 Salvage Chemotherapy for Non-Hodgkin's Lymphoma With a Combination of Mitoxantrone, Etoposide, Cisplatin, and Prednisolone. **T. Ohnoshi, K. Hayashi, H. Ueoka, T. Yamane, K. Ueno, M. Murashima, A. Tada, M. Yoshida, and I. Kimura.** Okayama University Medical School, Japan

643 Lack of Benefit of Adjunctive Chemotherapy in Stage I Malignant Melanoma: A Southwest Oncology Group Study. **B. L. Tranum, D. Dixon, J. Quagliana, J. Neidhart, S. P. Balcerzak, J. H. Costanzi, C. J. Fabian, B. Neilan, T. Maloney, R. M. O'Bryan, and C. Groppe.** Southwest Oncology Group

645 Total-Body Irradiation and High-Dose Teniposide: A Pilot Study in Bone Marrow Transplantation for Patients With Relapsed Acute Lymphoblastic Leukemia. **R. A. Krance, S. J. Forman, and K. G. Blume.** City of Hope National Medical Center, Duarte, California

649 Phase II Trial of Aminothiadiazole in Previously Treated and Untreated Patients With Advanced Colorectal Carcinoma: An Illinois Cancer Council Trial. **G. Y. Locker, J. Khandekar, S. Krauss, H. Reisel, T. Hoeltgen, J. Wolter, M. Haid, R. Hoffman, R. Blough, and C. Johnson.** Illinois Cancer Council

651 Hickman Catheter Clots: A Common Occurrence Despite Daily Heparin Flushing. **A. J. Anderson, S. H. Krasnow, M. W. Boyer, M. L. Raucheisen, C. E. Grant, O. R. Gasper, J. K. Hoffmann, and M. H. Cohen.** Veterans Administration Medical Center, Washington, DC

655 Treatment of Favorable-Prognosis Nonseminomatous Testicular Germ Cell Tumors With Etoposide, Cisplatin, and Reduced Dose of Bleomycin. **M. Brada, A. Horwich, and M. J. Peckman.** Institute of Cancer Research and The Royal Marsden Hospital, London and Surrey, England

CLINICAL TRIALS SUMMARIES

657 Phase II Trial of Idarubicin in Patients With Pancreatic Cancer. **A. Mittelman, G. B. Magill, V. Raymond, C. N. Sternberg, E. W. Cheng, P. B. Sordillo, and C. W. Young.** Memorial Sloan-Kettering Cancer Center, New York, New York

659 Treatment of Refractory Non-Hodgkin's Lymphomas of Unfavorable Histology With Teniposide, Cytarabine, and Cisplatin. **A. Tseng, Jr, C. Jacobs, C. N. Coleman, S. J. Horning, B. J. Lewis, and S. A. Rosenberg.** University of California San Francisco Medical Center

661 Phase II Trial of Etoposide in the Management of Advanced or Recurrent Mixed Mesodermal Sarcomas of the Uterus: A Gynecologic Oncology Group Study. **R. E. Slayton, J. A. Blessing, P. J. DiSaia, and W. A. Christopherson.** Gynecologic Oncology Group

663 Phase II Trial of Ifosfamide and Mesna in Advanced Colorectal Cancer. **N. Kemeny, B. Reichman, J. Dougherty, R. Lipperman, E. Cheng, and Physicians from the Community Clinical Oncology Program.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

LETTERS

665 Synthetic Enkephalin Analog in the Treatment of Cancer Chemotherapy-Induced Vomiting. **P. Lissoni, S. Barni, S. Crispino, G. Tancini, F. Paolorossi, L. Ferri, and G. Delitala.** San Gerardo Hospital, Monza, Milan, Italy

666 "Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?" **H. W. Bruckner.** The Mount Sinai School of Medicine, New York, New York

668 *Reply.* **B. Leyland-Jones.** National Cancer Institute, Bethesda, Maryland

668 Drug Eruption Localized to Previously Irradiated Skin. **S. J. Vukelja, W. D. James, and R. B. Weiss.** Walter Reed Army Medical Center, Washington, DC

669 High-Dose (200 mg/m^2) Cisplatin-Induced Neurotoxicity in Primary Advanced Ovarian Cancer Patients. **P. B. Panici, S. Greggi, G. Scambia, P. Di Roberto, S. Iacobelli, and S. Mancuso.** Università Cattolica S. Cuore, Rome, Italy

671 ANNOUNCEMENTS

EDITORIAL

Patient Accrual to Clinical Trials[1]

Michael A. Friedman[2,*]

Clinical research is often an empirical discipline based on inductive scientific principles. The results of a clinical experiment performed in a population of limited size are presumed to be applicable to all patients with a like type and stage of disease. The article in this issue of *Cancer Treatment Reports* by Hunter et al (1) describes the demography of patients accrued by the Community Clinical Oncology Program (CCOP) to National Cancer Institute (NCI)-sponsored clinical trials between August 1984 and November 1985. This experience raises important issues for both the design and interpretation of multi-institutional cancer clinical trials in the United States today.

Hunter et al describe the number and type of patients accrued, the types of studies they entered, and some factors that limited patient and physician participation. The descriptive information provided is based on a selected subset of all 60 CCOP grant holders. Forty-six CCOPs with relatively complete new-patient logs provided information on 44,156 patients identified over a 15-month period. Of 16,996 patients who were technically protocol-eligible, only 19% actually entered an NCI-sponsored study. These 3229 patients were not considered to be entirely representative of cancer patients in this country (differing from the SEER reference population) in that they generally were whites with advanced disease and had a relatively low median age of 59 years.

This article highlights several peculiarities and inefficiencies in the national cooperative clinical trials mechanism that are worth examination. Why is an $8,000,000 CCOP (and a $55,000,000 Cooperative Group) program not capable of generating larger numbers of patients with specific types of malignancies to resolve important scientific dilemmas confronting oncologists? Ideally, a national clinical trials mechanism would attract study populations with diseases of interest in an efficient manner.

This CCOP experience demonstrated that most new patients encountered could not be entered on protocol since they failed to meet eligibility criteria. The available protocols had been composed with the conventional clinical trial's wisdom. NCI-sponsored trials usually concentrate on a homogeneous group with optimal performance and organ function status. Traditionally, new therapies are initially tested in the "best possible" patient population, and there are many cogent arguments for these restrictions. Since all treatments have attendant toxic effects, the therapeutic index is thought to be greater in good-risk patients. Moreover, although highly effective therapies work even in patients with advanced but sensitive diseases (like testis cancer or Hodgkin's disease), intimations of effectiveness can be gained in selected patients with more refractory illnesses. For example, women with dermal metastases of melanoma are far more likely to respond and live longer than men with liver and bone metastases. Restricting eligibility for clinical studies can enhance the chances of identifying promising therapies. However, there needs to be a balance between the desire to be discriminatory and restrictive for scientific purity and the desire to address a population representing the average patient. Clearly, in the "real world" as represented by the CCOPs, only a minority of patients fit the protocol-dictated entry demands.

The functional characteristics of protocol patients are of prognostic importance. There is usually a decay in the apparent efficacy (response rate and/or survival time) of a treatment program over time. As first reported from Cancer Centers, then replicated by cooperative groups and exported to the general oncology practice community, there is usually a consistent decrement in observed results. While there are many possible explanations for this phenomenon, the restrictive eligibility criteria of smaller studies may be a major factor. A large number of CCOP patients could not be entered in

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clinical trials because they were not protocol-eligible. Therefore, the generalizability of clinical studies may in some cases be sacrificed to the interests of strict scientific discipline.

An equally troubling problem is the absolute number of patients recruited to available studies. One of the expectations engendered by the CCOP program was that by increasing community access to clinical trials more rapid answers to research questions could be achieved. It is estimated that 20% of all patients entered into NCI-sponsored studies come via the CCOP route. Judging by the data supplied, there were an average of 770 new patients screened by each CCOP per year, and each entered 145 patients on clinical trials per year. Assuming that most CCOPs evaluated patients with common adult malignancies, protocols should have been available for most patients and a capture rate higher than 31% should have been possible.

This article also questions why there are not sufficient patients available for the highest priority trials. Given the accumulating data of therapeutic benefit, it seems most unfortunate that so few CCOP patients were entered in adjuvant studies. Adjuvant studies for patients with common tumors (eg, breast or colorectal cancer) are of high scientific and clinical interest. However, only a small fraction of the potentially available adjuvant patients were actually entered in the study. Examining national trends reveals that only about 1%–1.5% of available breast cancer, 0.5% of rectal cancer, and 1% of colon cancer patients are actually recruited into such studies. Clearly, the CCOPs as currently constituted are not likely to enhance more general participation in these trials.

Finally, we have relatively little information as to what are the crucial impediments to entering a higher proportion of eligible patients into clinical studies. The CCOP logs demonstrated a high level of physician noncooperation. In roughly one-half of the patients, physician refusal to participate was noted, and patient refusal was noted in an additional one-third of patients. Speculation as to what financial, psychological, or other factors influenced the physicians' and patients' decisions not to enter a study is sterile. An insightful analysis of reasons for nonparticipation would be methodologically difficult, but is manifestly necessary. The challenge with so many promising therapeutic options available is to efficiently resolve clinical dilemma and effectively identify new therapies. The information provided by Hunter et al (1) begins to define the magnitude of the problems encountered. Only a more thorough investigation will lead to the remedies which are required.

REFERENCE

1. HUNTER CP, FRELICK RW, FELDMAN AR, ET AL. Selection factors in clinical trials: results from the Community Clinical Oncology Program physician's patient log. Cancer Treat Rep 71:559–565, 1987.

Selection Factors in Clinical Trials: Results From the Community Clinical Oncology Program Physician's Patient Log[1]

Carrie P. Hunter,* Robert W. Frelick, Allen R. Feldman, Anne R. Bavier, Wilma H. Dunlap, Leslie Ford, Donald Henson, Dorothy Macfarlane, Charles R. Smart, Rosemary Yancik, and Jerome W. Yates[2]

Between August 1984 and November 1985, data concerning 44,156 newly diagnosed cancer patients were entered in the Community Clinical Oncology Program Physician's Patient Log. Forty percent (17,773 patients) had a National Cancer Institute-approved protocol available for site and stage of disease. Fifty-six percent of patients with a protocol available were clinically eligible and 19% were entered on protocol. Thus, one-third of clinically eligible patients were entered in the study. Age was a major factor influencing eligibility and entry on study. Eighty percent of patients < 25 years of age with a protocol available were clinically eligible for clinical trials participation, and 58% were placed on protocol. Patients ≥ 65 years of age with a protocol available were less likely to be clinically eligible (48%) or placed on study (14%). A physician's preference for an alternate treatment and patient refusal for protocol treatment were also important reasons that clinically eligible patients were not registered on protocol. Selection for clinical trials participation affects the characteristics of patients under study and may have an impact on the ability to generalize the results of clinical trials. [Cancer Treat Rep 71:559–565, 1987]

Over the past 30 years, the development of clinical trials has progressed from research on advanced cancers using single or combined chemotherapy to the development of combined modality treatments, adjuvant approaches, and the investigation of chemotherapy dosage scheduling relationships for treating cancer patients. The progress of clinical trials research is reflected by the improved survival which has been demonstrated in patients with acute lymphocytic leukemia (ALL) (1), Hodgkin's disease (1,2), testicular cancer (3), choriocarcinomas (4), and, more recently, women with premenopausal breast cancer (5,6).

Reports from such clinical trials have proven useful in disseminating information concerning optimal cancer management practices to the community where > 80% of cancer patients receive care. During the past decade, increasing numbers of university-trained community oncologists have entered patients into cancer treatment research clinical trials. Studies by the Eastern Cooperative Oncology Group (ECOG) (7,8) and the Northern California Oncology Group (9) demonstrate that clinical research can be successfully conducted in community settings. Presently, this important national resource of well-trained oncologists practicing in the community setting enter approximately one-third of all cancer patients registered into National Cancer Institute (NCI)-sponsored clinical trials.

In 1983, the Community Clinical Oncology Program (CCOP) was implemented in 34 states and 62 US communities by the Division of Cancer Prevention and Control, NCI. The CCOP was designed to make available to community oncologists and their patients clinical research protocols representing state-of-the-art cancer patient management. Through community participation in clinical trials research, it was envisioned that the CCOP would increase information dissemination and diffusion of protocol-like management to cancer patients not on protocol. It was hypothesized that increasing community access to clinical trials would improve cancer care and provide more rapid answers to important research questions. The success of the CCOP has been demonstrated by its contribution of approximately 20% of all cancer patients entered in clinical treatment trials and the increased numbers of physicians and other health care professionals engaged in clinical research. This paper will address additional benefits of the CCOP: (*a*)

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[2] Divisions of Cancer Prevention and Control (*C. P. Hunter, R. W. Frelick, A. R. Feldman, A. R. Bavier, W. H. Dunlap, L. Ford, D. Henson, C. R. Smart, R. Yancik,* and *J. W. Yates*) and Cancer Treatment (*D. Macfarlane*), National Cancer Institute, Bethesda, MD.

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the unique and useful information provided on the characteristics of the cancer patient population from which patients entered in clinical trials are derived; and (*b*) the process by which patients are recruited and selected for participation in clinical trials as determined from the CCOP Physician's Patient Log.

Limited information is available which describes the characteristics of the cancer population from which patients entered in clinical trials originate (7,10–12). A knowledge of the population represented in a clinical trial is important in interpreting study results. Patient selection for protocol entry may result in highly selected groups in all study arms of a clinical trial and diminish the generalizability of the information generated. The process of randomization in clinical trials represents an attempt to avoid selection bias in intraprotocol comparisons. Eligibility criteria of the protocols; physician preferences for alternate therapies and concerns over the ethics of randomization (13–15); and patient concerns over the risks, benefits, and quality of life offered through protocol participation (16,17) may influence the characteristics of the patient population accrued to studies. An attempt to understand these selection factors is important for the future planning of clinical research. This multiphasic process of patient selection for clinical trials will be discussed.

In the research reported here, participating CCOP physicians were required to maintain a patient log of all cancer patients who were potentially eligible for clinical trials participation. Eligibility status, entry on protocol, or reason for nonentry on protocol were recorded on each new cancer patient when an NCI-approved protocol was available for a specific site and stage of cancer. This report characterizes the CCOP patients who were available for protocol studies and identifies some of the selection factors which influenced patient participation in clinical trials.

MATERIALS AND METHODS

The CCOP involved 187 hospital components, 63 physician's offices, and 37 group practices. The CCOPs affiliated with 14 cooperative groups and 17 comprehensive cancer centers (ie, research bases) to gain access to NCI-approved research protocols. Over 1000 community physicians involved in cancer treatment and approximately 300 data managers and nurse oncologists participated in the CCOP.

From August 1984 to November 1985, CCOP physicians and data management staff recorded the following data elements on the patient log for each cancer patient seen: a local patient identifier (not submitted to the NCI); the CCOP physician's identification number and specialty code; the date of entry on the log; patient initials; date of birth; sex; race-ethnicity; zip code of residence; patient status (newly diagnosed or established); primary cancer diagnosis; stage; and the availability of a CCOP protocol for the specific site and stage of cancer. For all patients for whom a protocol was available, the following information was recorded: the research base and protocol number; and the reason a patient was ineligible for a CCOP-approved protocol, or the registration of a patient on protocol, or the reason for nonentry of a clinically eligible patient on protocol. Treatment disposition was recorded for all patients with a protocol available.

The reliability of data submitted from 60 CCOPs was evaluated to determine the following: (*a*) discrepancies between the patient log and the available research base information of patients entered on protocol; and (*b*) the completeness of patient log entries. Logs with $>$ 10% missing data for certain key elements (stage; and for patients with protocols available: research base, protocol number, eligibility status, and treatment disposition); logs with underreporting of patients registered on protocol; and logs with incomplete reporting of quarterly data were considered incomplete.

Based on these criteria, data from 46 CCOPs qualified for use in developing this report. A comparison of the CCOPs included with those excluded showed no appreciable differences in data related to age, sex, race, or physician specialty. The distributions by disease site and stage of cancer were similar in both groups. Log entries from CCOPs in both groups had equal access to multidisease and specialty group research base protocols. The excluded CCOPs provided an incomplete denominator and were dropped because of probable systematic selection bias for those reported.

"Previously diagnosed" or "established" cancer patients, who accounted for 20% of all log entries, were excluded from this analysis. Eighty percent of patient log entries were "newly diagnosed" (44,156 patients); 40% had a cooperative group or cancer center research base protocol available for the particular site and stage of cancer. These 17,773 new cancer patients with protocols available form the basis of this report.

RESULTS

Demographics and Eligibility of Protocol-Available Patients

The median age of all new cancer patients with a protocol available was 64 years, which is similar to the median age of 65 years for incident cases reported to the NCI's Surveillance, Epidemiology, and End Results (SEER) Program (table 1). Patients determined to be clinically eligible and patients placed on protocol had lower median ages of 61 and 59 years, respectively. This reflects a progressive selection of persons for study

TABLE 1.—Median age of new cancer patients

	No. of patients	Median age (yrs)
SEER (1973–1977)	327,627	65
CCOP		
Protocol available	17,674	64
Clinically eligible	9508	61
On protocol	3242	59

TABLE 2.—Sex and race distributions of new cancer patients

	SEER (1973-1977)	CCOP protocol available
Sex		
Total No.	327,627	17,746
Male	50%	43%
Female	50%	57%
Race		
Total No.	327,627	17,473
White	87%	93%
Black	8%	4%
Other ethnic groups	5%	3%

based on the age criteria of some protocols and other ineligibility criteria which exclude certain high-risk older patients from participation.

The sex and race of new cancer patients with protocols available are shown in table 2. While females accounted for one-half of incident cases reported to SEER, they accounted for 57% of CCOP patients with a protocol available. This difference in sex distribution was due to the higher proportion of breast cancer patients in the CCOP population (29% in CCOP vs 12% in SEER). Black, Hispanic, and other ethnic groups accounted for 7% of the total CCOP patients compared to 13% representation in SEER.

Of the CCOP patients, 42% had distant, 41% had regional, and 17% had local disease. One-half of CCOP colon and rectum cancer patients with a protocol available had distant disease compared to 23% in SEER. Distant disease was present in 22% of breast cancer patients compared to only 9% in SEER. The larger portion of cancer patients with advanced disease reported on the log is consistent with the types of patients usually seen by medical oncologists and hematologists who composed the largest proportion of the CCOP participating physicians. When CCOP protocols were available, the majority of patient log entries were made by medical oncologists and hematologists (77%) and radiation oncologists (16%). Surgical oncologists accounted for only 4% of patient log entries and 3% were entered by physicians from other disciplines.

Fifty-six percent of patients with a protocol available were clinically eligible for study and 19% were treated on protocol (fig 1). While the median ages of patients who were clinically eligible and on protocol were younger than protocol-available patients (table 1), these groups showed sex, race, and stage distributions similar to the protocol-available patients. Patients treated on protocol represented 34% of clinically eligible patients.

The proportion of protocol-available patients who were clinically eligible and placed on protocol decreased with age (fig 2). Eighty percent of patients < 25 years of age with a protocol available were clinically eligible for clinical trials participation, and 58% were placed on protocol. Acute myelogenous leukemia (AML), ALL, Hodgkin's disease, and non-Hodgkin's lymphoma accounted for 74% of patients < 25 years of age on protocol. Two-thirds of patients < 55 years of age were clinically eligible compared to 53% of patients 65–74 years of age and 40% of patients ≥ 75 years of age. For patients ≥ 75 years of age, only 7% were entered on protocol. Thus, in this elderly population, only 18% of clinically eligible patients were registered on study.

Site Distributions

Of the hematologic malignancies, protocols were available in 63% and 75% of ALL and AML patients, respectively. The majority of acute leukemia patients who had protocols available were clinically eligible for study (ALL, 85%; and AML, 68%). Of the eligible group, 87% of ALL and 67% of AML patients were entered in the study. Patients with Hodgkin's disease, non-Hodgkin's lymphoma, and myeloma had treatment protocols available to them less frequently (range, 27%–38%) than leukemia patients. Overall, approximately 50% of clini-

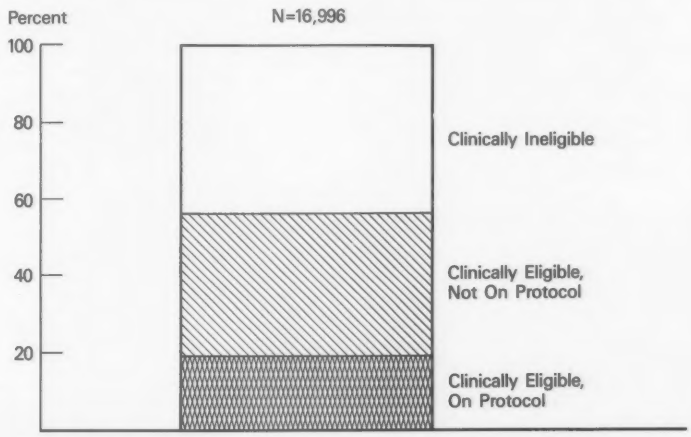

FIGURE 1.—Eligibility status of new cancer patients with protocols available.

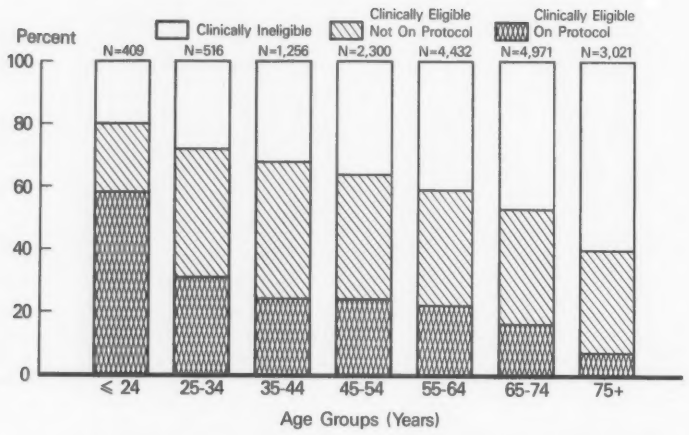

FIGURE 2.—Distribution by age groups of new cancer patients with protocols available.

cally eligible patients with hematologic malignancies were entered on study.

Sixty percent of breast and small cell lung cancer patients and approximately one-half of non-small cell lung, colon, and pancreatic cancer patients had a protocol available. Of the clinically eligible patients, 28% of breast cancer, 38% of small cell lung cancer, 32% of non-small cell lung cancer, 37% of colon cancer, and 29% of pancreatic cancer patients were entered on treatment protocols. Few protocols for head and neck cancer were available, and only 22% of the eligible patients were entered on study. Overall, 40% of the patients with solid tumor neoplasms had protocols available. Of these, one-half were clinically eligible, and one-third of the eligible patients were entered on protocol treatment.

Reasons for Protocol Ineligibility

The major reason patients were ineligible for clinical trials was protocol design (70%). Protocol design always includes specific ineligibility criteria (eg, exclusions due to elevated serum creatinine in patients under consideration for cisplatin administration and exclusions due to underlying cardiac disease in patients under evaluation for anthracycline chemotherapy). Poor performance status (14%), other malignancies (7%), and prior chemotherapy or radiation therapy (9%) accounted for the remainder of patients ineligible for clinical trials.

Reasons for Nonentry on Protocol

Reasons for nonentry on protocol of clinically eligible patients by sex and type of cooperative-group protocol are shown in table 3. The physician's preference for a specific treatment or alternative form of therapy was the predominant reason for nonentry in one-half of the eligible patients. This was especially true for phase II studies. Follow-up difficulty and concomitant medical problems precluded clinical trials participation in 10% of eligible patients and was a frequent reason for exclusion in nonadjuvant phase III and phase II studies. These latter observations reflect the advanced disease inherent in nonadjuvant phase III and phase II protocol designs.

One-third of the eligible patients refused to participate. Refusal was more common in females (34%) than males (29%) and was higher among patients offered adjuvant phase III protocols (37%) than nonadjuvant phase III (29%) or phase II (26%) studies. While females accounted for > 80% of the patients eligible for adjuvant trials (mostly breast cancer), there was little variation between genders by the type of study in reasons for nonparticipation in clinical trials. Experimentation (10%), toxicity (4%), and costs (2%) accounted for one-half of the specific reasons for patient refusal. However, experimentation was more often cited by females than males as a specific reason for nonparticipation in adjuvant studies.

Treatment Disposition

Nineteen percent of CCOP patients with a protocol available were entered on an NCI-approved protocol. Of patients entered on cooperative-group protocols, 43% were treated on nonadjuvant phase III, 31% on adjuvant phase III, 22% on phase II, and 4% on special studies (ie, pilot and quality of life studies). Thirteen percent of patients with a protocol available received no treatment. Fifty-two percent of the patients were treated with "other regimens" available to the CCOP physicians. Modified protocols (one in which the physician indicated that an equivalent or modified arm of a CCOP-approved protocol was administered) were used for 16% of patients with a protocol available. Twenty-five percent of patients eligible for adjuvant or nonadjuvant phase III protocols received a modified protocol regimen.

TABLE 3.—Clinically eligible patients: reasons for nonentry on protocol by sex and type of study*

	No. of patients	Reasons for nonentry on protocol (%)			
		Physician decision	Patient refusal	Follow-up difficulty and concomitant medical problems	Other
Total	5605	51	32	10	7
Male	2148	51	29	12	8
Female	3457	50	34	9	7
Adjuvant phase III	2263	50	37	8	5
Male	372	46	39	9	6
Female	1891	51	37	7	5
Nonadjuvant phase III	2209	48	29	14	9
Male	1126	48	28	15	9
Female	1083	48	30	12	10
Phase II	1133	56	26	10	8
Male	650	59	25	8	8
Female	483	54	27	11	8

*Special studies and center protocols excluded.

DISCUSSION

The results of clinical trials depend in part on: (*a*) the recruitment and participation of physicians in the clinical trials process (7,10–13); (*b*) the capabilities of the health care community to support clinical research; (*c*) the development and selection of suitable state-of-the-art protocols for cancers frequently occurring in the community (11); (*d*) the identification, recruitment, and eligibility of cancer patients available for study (10–12); and (*e*) the acceptance and participation of patients in clinical trials (7,10–12). This multiphasic clinical trials process evolves through several steps.

The CCOP experience was designed to include physicians from different specialities with a strong interest in participating in clinical research protocols. Approximately two-thirds of all CCOP physicians who were engaged in patient care actively evaluated cancer patients for clinical trials entry, and over half entered patients on treatment protocols. Patient selection for participation in clinical trials was first determined by the availability of a protocol, and second by the eligibility of the patient based on protocol-design criteria. Up to this point, factors in the selection process were independent of the patient's acceptance or refusal of clinical research. An understanding of those physician, protocol-specific, and patient-related factors which influence the types of patients ultimately entered on protocol should improve our ability to assess the validity and generalizability of future studies and enhance our knowledge and use of clinical trials results.

According to the CCOP log, over one-half of the patients with a protocol available were clinically eligible, and one-third of clinically eligible patients were entered on protocol. Age was a major factor influencing both eligibility and entry on study. Patients on protocol (median age, 59 years) were 2 years younger than those patients who were clinically eligible (median age, 61 years) and were 5 years younger than those patients who had a protocol available (median age, 64 years). While over half of cancer patients seen in the communities are > 65 years of age, they are less likely to go into clinical trials, even when clinically eligible. The lower age of eligible patients is the result of the age criteria imposed by some protocols (18,19) as well as the complexity of comorbid medical conditions in the elderly. After eligibility for a protocol has been established, physician, patient, and/or other medical factors enter into the decision-making process and determine the level of participation of patients in clinical trials.

The level of patient participation in clinical research was influenced by gender, type of protocol offered, and indirectly by stage of disease. Both males and females were less likely to engage in clinical research when early disease was present. Patient refusal was higher among females than males, and "experimentation" was more often a deciding factor in whether female patients entered adjuvant trials.

In an ECOG prevalence study comparing the level of participation of 104 community affiliates and 21 member institutions, both new and previously diagnosed cancer patients were surveyed (7). Advanced disease was recorded in 59% of member and 64% of community-affiliate patients. Sixty-six percent of cancer patients were not eligible for protocol treatment; however, half of these patients were not eligible due to a lack of an available protocol. Overall, two-thirds of the ECOG patients had a protocol available and one-half were judged to be clinically eligible. Clinically eligible patients were not registered on protocol because of a physician's preference for a specific treatment or alternate therapy (52%), patient refusal (19%), significant concomitant medical problems and anticipated difficulty with follow-up (17%), and other reasons (12%).

This analysis of new cancer patients from the CCOP log shows that 56% of newly diagnosed patients were clinically eligible for available protocols. Physician preference for an alternate form of therapy accounted for half of the clinically eligible patients not being registered on study. The ECOG survey reported similar results. Concomitant medical problems and anticipated difficulty in follow-up were cited on the log less often than in the ECOG survey as reasons for nonparticipation. Within the CCOP, advanced disease was less common, and nearly twice as many eligible patients refused to participate in clinical trials as refused in the ECOG survey. These differences between our study and the ECOG survey can be attributed to differences between the CCOP new cancer patient and the ECOG prevalence populations reported.

Of the hematologic malignancies, a majority of patients with acute leukemias were clinically eligible and entered on protocol. Hodgkin's disease and non-Hodgkin's lymphoma patients were less likely to have protocols available. When protocols were available, a greater proportion of younger patients were placed on study than patients $\geq$ 65 years of age, reflecting an age bias in the treatment selection process. It is also conceivable that the less frequent entry on protocol in these two disease sites is also related to a perception in the oncology community that good "standard" treatment is available, resulting in less than enthusiastic participation in available clinical trials.

Ethical considerations related to randomized clinical trials continue to inhibit some clinicians from entering patients on protocol studies (13–15,20). Physician conflicts between their responsibility to provide patients with the best treatment available and their responsibility to the larger community to support scientifically sound research continue to cause difficulty in the doctor-patient relationship. The enrollment of a patient on an experimental study is viewed by some physicians as a compromise of their moral responsibility to look after the welfare of the individual patient. The high percentage of clinically eligible patients who are excluded from clinical trials participation due to a "physician's decision" may in part reflect this ethical dilemma, disapproval of the protocol design, limited support to conduct the treatment plan, or limited time to adhere to protocol guidelines while meeting the demands of a busy office practice.

The large number of eligible patients excluded from protocol participation due to a physician's decision and patient refusal raises questions on the adequacy of the selection and recruitment process in clinical research. Better methodologies are needed to implement multi-institutional studies, increase the commitment of physicians to clinical trials, improve patient understanding of research, validate clinical trials results, and rapidly complete and disseminate treatment information to the scientific community.

In conclusion, the CCOP log breaks into component parts the complex process by which patients are entered in clinical trials. The selection, recruitment, and entry of cancer patients into protocols involves an understanding of the protocol requirements and physician and patient factors which can influence protocol participation. The validity and generalizability of clinical trials must take into account this complex selection process.

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Synergistic Effect of Recombinant Interleukin-2 on the Inhibition of Growth of Adenocarcinoma 755 by Mitomycin[1,2]

Masaaki Iigo,[3,*] Masanori Sakurai,[4] Nagahiro Saijo,[4] and Akio Hoshi[3]

The increase in life-span (ILS) of tumor-bearing mice caused by recombinant interleukin-2 (RIL-2) was studied in the solid tumor adenocarcinoma 755 system. With long-term treatment (Days 5–12), RIL-2 (10^5 U/mouse) showed a weak effect (24% ILS), but short-term RIL-2 treatment (Days 5–8 or 9–12) had hardly any effect. Mitomycin, at 2 mg/kg (maximum nontoxic dose), caused 35% ILS with Days 5–8 treatment and only 12% ILS with Days 9–12 treatment. Sequential treatment with mitomycin (Days 5–8) and RIL-2 (Days 9–12) markedly enhanced antitumor activity (88% ILS). This value is significantly greater than that of mitomycin alone or RIL-2 alone ($P < 0.01$). Furthermore, mitomycin followed by RIL-2 markedly augmented killing activity of spleen cells that are cytotoxic in vitro. These results indicate that RIL-2 markedly affects the inhibition of growth of a tumor treated with an antitumor agent. [Cancer Treat Rep 71:567-570, 1987]

Interleukin-2 (IL-2) is a secretory product of activated T-lymphocytes. By recombinant DNA technology, human interleukin-2 (RIL-2) is now produced in bacteria (1). RIL-2 together with lymphokine-activated killer (LAK) cells mediates regression of established pulmonary metastases from a variety of syngeneic sarcomas in C57BL/6 mice but RIL-2 alone has little effect (2). On the other hand, the antitumor agent mitomycin has a marked effect on the inhibition of tumor growth, but it depresses the activity of natural killer (NK) cells (3) and maybe also the generation of host lymphoid cells with antitumor activity. Therefore, expansion of endogenous lymphoid cells is needed to maintain host defense. Recently, Chang et al (4) reported that systemically administered RIL-2 results in the generation of spleen cells in vivo that are cytotoxic in vitro. Moreover, RIL-2 has been found to stimulate the proliferation (5) and activity (6) of NK cells. Therefore, we conducted a study to determine whether antitumor activity against a solid tumor is increased by RIL-2 or not when administered with mitomycin.

MATERIALS AND METHODS

Mitomycin was purchased from Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan. RIL-2 (sp. 1×10^7 U/mg of protein) was supplied by Biogen S.A. Co, Switzerland, and Shionogi and Co, Osaka, Japan.

Groups of six or 12 male BDF_1 mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing 20–23 g were used. The solid tumor adenocarcinoma 755 (5×10^5 cells/mouse) was implanted sc into the right thigh on Day 0. The tumor had been maintained by sc transfer every 12 days in C57BL/6 mice in our laboratory. The mice were given mitomycin (iv) and/or RIL-2 (sc [left side]) in four consecutive daily treatments in the advanced tumor system.

The growth of sc implanted adenocarcinoma 755 was monitored by caliper measurement of perpendicular diameters, and tumor volume (mm^3) was calculated by the formula $1/2 \times$ (major diameter in mm) $\times$ (minor diameter^2 in mm^2). Survival times (days) of treated animals were compared with those of untreated controls, and the mean increase in life-span (ILS) was calculated.

To determine cell-mediated cytotoxicity, $Na_2{}^{51}CrO_4$ (Amersham, England) -labeled target cells (YAK-1, B-16, and F-10 melanoma) were incubated with spleen cells of mice treated with mitomycin at 2 mg/kg (iv) and/or RIL-2 at 10^5 U/mouse (sc) in Linbro 96-well microtiter plates (Linbro Scientific Co, USA). The details of the technique have been described previously (3).

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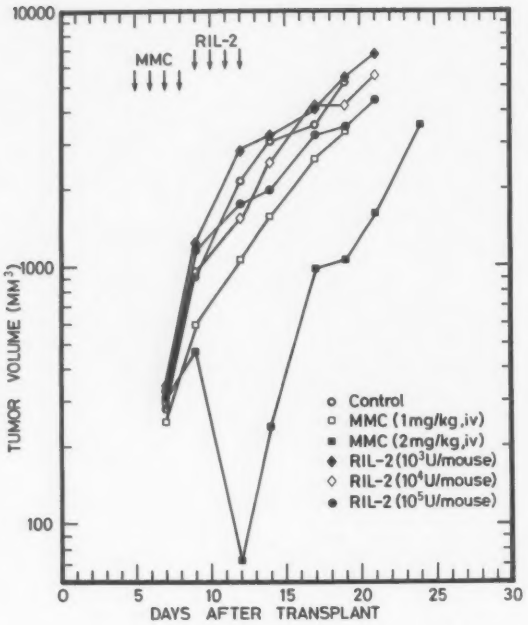

FIGURE 1.—Effect of mitomycin or RIL-2 on the growth of advanced solid tumor (adenocarcinoma 755). MMC = mitomycin.

Data were analyzed for significance by the two-tailed Student's t-test.

RESULTS AND DISCUSSION

The half-life of IL-2 in the serum after sc injection is longer than after iv or ip injection (7); RIL-2 at 10^5 U/mouse results in augmentation of the cytotoxicity of spleen lymphocytes against syngeneic B-16 melanoma cells (8) and showed a slight inhibitory effect on the growth of adenocarcinoma 755 when administered by sc injection for 4 consecutive days beginning on the fifth day after tumor transplant (fig 1). RIL-2 (10^5 U/mouse) in long-term treatment (Days 5–12) caused a significant prolonged survival of tumor-bearing mice (24% ILS, $P < 0.01$ versus control), but not with the short-term treatment (Days 5–8 or 9–12) (table 1). Mitomycin at a dose of 2 mg/kg, which was the maximum nontoxic dose in this schedule, caused marked inhibition of tumor growth temporarily (fig 1) and 35% ILS with Days 5–8 treatment, but only 12% ILS with Days 9–12 treatment (table 1). All mice treated with mitomycin at 4 mg/kg died within 15 days after transplant.

Mitomycin depresses the activity of NK cells in normal mice (3). In tumor-bearing mice, the cytotoxicity of spleen lymphocytes was markedly decreased (table 2). However, mitomycin enhanced the activity in tumor-bearing mice but the activity was lower than that of normal mice. This might suggest that suppressor T-cell

TABLE 1.—Effect of interleukin-2 (RIL-2) on the antitumor activity of mitomycin against adenocarcinoma 755

	Days of treatment	Growth rate on Day 14 (treated/control, %)	Days of survival Mean ± SE (No. of mice)	Treated/ control (%)
Control		100*	18.5 ± 0.7 (12)	—
Mitomycin, 2 mg/kg, iv	5–8	9	25.0 ± 2.1 (12)	135†
Mitomycin, 2 mg/kg, iv	9–12	66	20.8 ± 2.0 (6)	112
RIL-2, 10^5 U/mouse, sc	5–8	73	18.9 ± 0.6 (12)	102
RIL-2, 10^5 U/mouse, sc	9–12	36	20.9 ± 0.7 (8)	113
Mitomycin, 2 mg/kg, iv RIL-2, 10^5 U/mouse, sc	5–8 5–8	5	23.3 ± 1.4 (6)	126
Mitomycin, 2 mg/kg, iv RIL-2, 10^5 U/mouse, sc	5–8 9–12	4	34.7 ± 1.7 (12)	188‡
RIL-2, 10^5 U/mouse, sc Mitomycin, 2 mg/kg, iv	5–8 9–12	64	22.0 ± 0.5 (6)	119
RIL-2, 10^5 U/mouse, sc	5–12	21	22.9 ± 1.1 (12)	124†

*Tumor volume of control was 2760 ± 440 (SE) mm^3.
†Significantly different value, $P < 0.01$ vs control.
‡Significantly different value, $P < 0.01$ vs mitomycin alone.

TABLE 2.—Augmentation of killing activity of spleen lymphocytes by mitomycin and RIL-2 in tumor-bearing mice

Experiment No.	Treatment*	Days of treatment	Target cells† YAC-1	B-16	F-10
I	Non-tumor-bearing mice		18.8 ± 0.8‡	7.4 ± 0.3	1.9 ± 0.5
	Tumor-bearing mice (control)		12.2 ± 0.6	4.6 ± 1.5	1.2 ± 0.5
	Mitomycin (2 mg/kg)	Days 5–8	18.2 ± 1.1	4.4 ± 0.8	0.5 ± 0.4
	RIL-2 (10^5 U/mouse)	Days 5–8	28.0 ± 1.2	21.8 ± 1.2	6.8 ± 0.9
	Mitomycin + RIL-2	Days 5–8	32.2 ± 2.3	28.1 ± 2.8	6.9 ± 0.3
II	Non-tumor-bearing mice		15.9 ± 2.0	7.9 ± 1.6	2.0 ± 0.5
	Tumor-bearing mice (control)		4.8 ± 0.6	0.0	0.4 ± 0.2
	Mitomycin	Days 5–8	10.4 ± 0.8	2.2 ± 1.4	1.3 ± 0.2
	RIL-2	Days 5–8	9.3 ± 0.9	5.4 ± 0.1	0.1 ± 0.2
	Mitomycin + RIL-2	Days 5–8	15.6 ± 0.5	4.9 ± 1.0	0.7 ± 0.8
	Mitomycin / RIL-2	Days 5–8 / Days 9–12	20.8 ± 0.8	33.0 ± 0.5	5.6 ± 0.7
	RIL-2 / Mitomycin	Days 5–8 / Days 9–12	7.6 ± 1.4	8.1 ± 1.7	1.6 ± 0.4

*Cytotoxicity against target cells was determined on Day 9 (I) and Day 13 (II) after tumor transplant. Three or 6 mice/group were used.

†Murine spleen cells (1×10^6/well) were assayed for cytotoxicity against target cells (1×10^4/well).

‡% Cytotoxicity, mean ± SE.

activity in the spleen is inhibited by mitomycin, like cyclophosphamide (9). RIL-2 stimulates proliferation of NK cells in normal mice (5); it also augmented killing activity of host lymphocytes with antitumor activity in tumor-bearing mice.

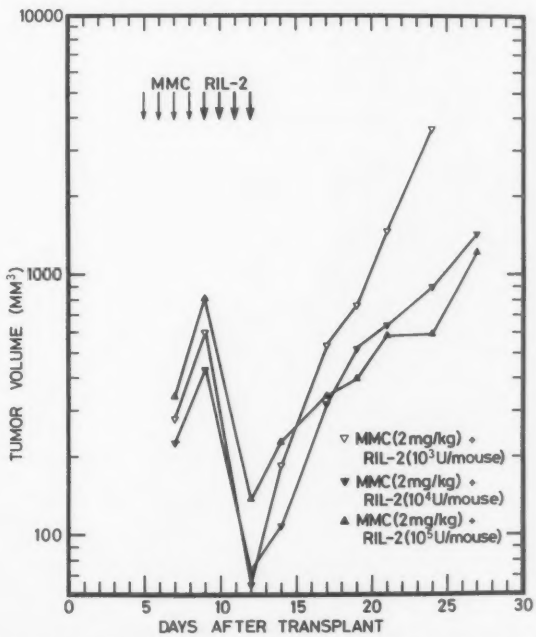

FIGURE 2.—Combination effect of mitomycin and RIL-2 on the growth of advanced solid tumor (adenocarcinoma 755). MMC = mitomycin.

Sequential treatment with mitomycin (Days 5–8) and RIL-2 (Days 9–12) markedly prolonged survival (88% ILS), with a significant difference from mitomycin or RIL-2 alone ($P < 0.01$). After treatment with mitomycin (Days 5–8) followed by RIL-2 at a dose of 10^4 or 10^5 U/mouse (Days 9–12) tumor growth was slower than with mitomycin alone (fig 2), but RIL-2 at 10^3 U/mouse did not affect the tumor growth. Simultaneous treatment with mitomycin and RIL-2 (Days 5–8) and sequential treatment with RIL-2 (Days 5–8) and mitomycin (Days 9–12) showed no significant difference from mitomycin alone (table 1).

RIL-2 slowed the tumor growth when administered after an antitumor agent. Posttreatment with RIL-2 could markedly augment killing activity of spleen cells on the 13th day after tumor transplant compared with any other treatments as shown in table 2. Thus, the order of the two drugs affected the killer cells' activity and the most effective treatment schedule was the mitomycin followed by RIL-2. These results indicate that posttreatment with RIL-2 is very important for enhancement in immunochemotherapy. Further studies should be conducted to determine what kinds of cells are generated after chemotherapy.

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Influence of Daunorubicin and Cytarabine Sequencing on the Outcome of Therapy in Acute Myelogenous Leukemia: A Randomized Trial[1]

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Ninety-seven patients ≤ 70 years of age with previously untreated primary acute myeloblastic leukemia were randomly treated with either the DAT or TAD regimen: daunorubicin (70 mg/m²/day) administered on Days 1-3 (DAT) or 5-7 (TAD) of a 7-day sequence consisting of cytarabine (200 mg/m²/day) and 6-thioguanine (200 mg/m²/day). Complete responders received consolidation, maintenance, and final intensification over 14 months using mostly the same drugs as during induction administered in the same sequence. The regimens did not significantly differ from each other with regard to toxicity or efficacy. Complete remission rate was 80% in the two groups, and median duration of complete remission was 549 days with DAT and 518 days with TAD. [Cancer Treat Rep 71:571-574, 1987]

During the last decade, considerable improvement has been achieved in the treatment of acute myeloid leukemia (AML). Combinations of cytarabine (araC) with daunorubicin (DNR) and 6-thioguanine (TG) have increased complete remission (CR) rates to 50%–80% (1). However, although sequencing of administration of araC and DNR has been shown to be important for in vitro cell kill (2), better killing being achieved when DNR is administered following araC, CR rates > 80% have been reported in studies from one center when DNR was administered at the beginning (DAT) as well as at the end (TAD) of an araC-TG sequence (3,4). No randomized comparison of TAD versus DAT has been reported, to our knowledge. Furthermore, emphasis has been placed on the timing of administration of the different drugs to achieve maximal in vitro cell kill (2,5,6) and thus potentially even higher CR rates in vivo.

We report here the results of a randomized comparison between pharmacologically timed TAD and DAT sequences. Based on reports already published or in press at the time of initiation of this study suggesting the interest of early consolidation (7) and late intensification (8) in addition to maintenance, we decided to treat our CR patients with intensive consolidation, relatively short maintenance, and late intensification chemotherapy. The consolidation and intensification courses were sequenced in the same way as the induction course received by the same patient to analyze a possible effect of sequencing of araC-TG and DNR on CR duration as well as CR rate.

MATERIALS AND METHODS

Patients

Between October 1979 and May 1982, 97 patients were randomized in this trial. All of them had newly diagnosed and previously untreated primary AML and were < 70 years of age. During this trial, 19 patients < 70 years of age with AML were excluded from the study for the following reasons: eight had a past history of myelodysplastic syndrome for > 3 months before AML was diagnosed; nine were in critical condition and died before chemotherapy; one had cardiac contraindication to anthracyclines; and one refused therapy. AML was diagnosed and classified according to French-American-British (FAB) criteria (9).

Chemotherapy Schedule

Induction.—DAT consisted of DNR (70 mg/m² by iv push on Days 1-3), araC (50 mg/m² by iv push administered 12 hours after DNR and immediately followed by 150 mg/m² iv as a 12-hour infusion on Days 1-7), and TG (200 mg/m² orally given just after DNR on Days 1-7).

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TAD was identical to DAT except that DNR was administered on Days 5–7 of the araC-TG sequence. Patients with a wbc count > 1000/mm^3 on Day 7 of treatment received araC at the same dose as on Days 1–7 for 2 additional days. If no CR was achieved, a second course of the same induction chemotherapy regimen was administered.

Consolidation.—Consolidation was started as soon as CR was achieved. In the DAT arm, consolidation consisted of courses given once a month for 3 months. Courses consisted of DNR at a dose of 60 mg/m^2 on Days 1 and 2 (Courses 1 and 3) or cyclophosphamide (CTX) at a dose of 800 mg/m^2 iv in a 3-hour infusion on Day 1 (Course 2) consisting of araC and TG administered at the same dose and in the same schedule as during induction from Day 1 to Day 5. Consolidation therapy in the TAD arm differed from that of the DAT arm only by the administration of DNR on Days 4 and 5 and by the administration of CTX on Day 5 of the araC-TG sequence.

Maintenance therapy.—Maintenance was the same in both arms and consisted of courses given once a month for 8 months. Courses consisted of CTX (600 mg/m^2 iv on Day 1), mitoguazone (400 mg/m^2 iv on Day 1), and araC (200 mg/m^2/day in two sc injections on Days 1–5).

Final intensification.—Final intensification was initiated 1 month after the last maintenance course and consisted of courses given once a month for 3 months. The first course was identical to the first consolidation course in each arm except that DNR was given on Day 1 or Day 5 only. The second course consisted of vincristine (2 mg/m^2 iv on Day 1), 6-mercaptopurine (500 mg/m^2 orally on Days 1–5), methotrexate (7.5 mg/m^2 im on Days 1–5), and prednisone (100 mg/m^2 orally on Days 1–5). The third intensification course consisted of carmustine (100 mg/m^2 iv on Day 1), CTX (800 mg/m^2 iv on Day 1), vincristine (1 mg/m^2 on Day 2), and araC (200 mg/m^2 iv administered in the same schedule as during induction and consolidation on Days 3–7).

Supportive Care

During induction, all patients were managed in reverse isolation rooms and received prophylactic intestinal decontamination using nonabsorbable antibiotics and nystatin. Empiric parenteral antibiotherapy was initiated as soon as fever was > 38.5°C and was subsequently adapted to isolated organisms. Prophylactic platelet transfusions were administered to keep the platelet count > 20 × 10^7/L. Hyperuricemia was prevented by alkaline perfusions and oral allopurinol at a dose of 300 mg/day. In case of disseminated intravascular coagulation, 1 mg/kg/day of heparin was administered as a continuous infusion.

Evaluation

CR was documented according to Cancer and Leukemia Group B criteria (10) on a marrow aspirate performed prior to first consolidation. Partial remissions were considered as failures. Failures were classified according to Preisler (11). In brief, resistant disease was diagnosed in patients who had blastic regrowth after chemotherapy or who died beyond 1 week after completion of chemotherapy with leukemic blast cells still present in their marrow at the time of death. Other failures consisted of patients who either died early, within 1 week after completion of chemotherapy, or later, during aplasia but with < 5% blast cells in their marrow at the time of death. CR duration was calculated from the documentation of CR to relapse or date last seen. Survival was calculated from diagnosis to death or date last seen. Three patients, all of them randomized in the TAD arm, received allogeneic bone marrow transplantation from an HLA-identical sibling in early CR and were excluded from analysis of CR duration and survival at the time of transplant. No patient was lost to follow-up and data were reported in early 1986.

Statistical Methods

Before beginning the study, it was determined that randomization of 66 eligible patients in each arm would be necessary to demonstrate an improvement in CR rate. It was necessary to improve the average CR rate from 65%, which was reported in various series where DNR was administered at the beginning of an araC or araC-TG sequence (1), to 85%, a CR rate expected from TAD according to the first report regarding this regimen (4), based on a one-tailed test of difference in CR rate at a 0.05 significance level with a power of 0.80 (12). We subsequently decided to close the study after only 97 eligible patients had been entered because the first interim analysis done at that point and discussed below indicated that it was very unlikely that the statistical difference in CR rate between DAT and TAD would ever be demonstrated, mainly because of a better than anticipated CR rate in the control DAT arm.

Remission duration and survival curves were calculated using the method of Kaplan and Meier (13). Signification of differences between survival curves was analyzed using the log-rank test (14).

RESULTS

Initial characteristics of patients randomized in the DAT and TAD arms are indicated in table 1, which shows that clinical and biological parameters were similar in both groups except for the FAB type of AML: patients in the DAT group had more FAB M3 and M5 AML, while patients in the TAD group had more FAB M1 and M2 AML. Toxicity and efficacy of DAT and TAD are shown in table 2. Hematologic toxicity of the induction course was not statistically different in the two arms. Hematologic toxicity was also observed in both arms in patients achieving CR during consolidation and final intensification, but marrow hypoplasia was of short duration and only occasionally were patients hospitalized. However, one patient in the DAT

arm died from infection after the second consolidation while in CR. Nonhematologic toxicity in DAT and TAD occurred mainly in the digestive tract and was only mild to moderate. CR rate was 80% in both arms (table 2); there was an approximate 95% two-sided confidence limit for the difference in CR rates ranging from 15% favoring the TAD regimen to 15% favoring the DAT regimen. Ten patients receiving DAT and nine receiving TAD received 9 days of araC based on the Day-7 wbc count. The CR rate for these patients was not statistically different from that of patients receiving the 7-day treatment. None of the patients on the DAT arm and three on the TAD arm achieved CR after two induction courses. Failures were due to toxic deaths, related to infection in all patients, rather than to resistant disease (table 2). Remission duration and overall survival were not statistically different in the two arms or in patients who received 9 days of araC during induction by comparison to patients receiving the 7-day regimen. At the time of analysis, 12 of the 37 complete responders on the DAT arm and eight of the 38 complete responders who did not receive bone marrow transplantation on the TAD arm were surviving relapse-free.

DISCUSSION

The randomized study presented here shows no significant differences in efficacy or in toxicity between pharmacologically timed DAT and TAD sequences for the initial treatment of AML, although the study is not large enough to exclude differences of up to 10%–15% in CR rate. This confirms results obtained by Omura (15) in a nonrandomized trial. As already emphasized, there were some differences between our two groups of patients regarding some FAB types of AML. However, these differences were certainly not of enough magnitude to influence the overall results of the trial, since analysis did not show any statistically significant difference in CR rate and survival between the involved FAB types of AML in our series (data not shown), a result already found by others (1). Our 80% CR rate, found in both arms of the study, is similar to what has been recently found in a similar highly selected AML patient population (16) and does not allow conclusions to be drawn regarding the timed use of DNR, TG, and araC. A randomized study comparing timed and simultaneous use of DNR, araC, and TG would be necessary to test the clinical importance of the timing of administration of the three drugs. Most induction failures in our patients were due to death from infection and one could conclude that our induction regimens using higher than usual doses of DNR had excessive toxicity. It is probable, however, that resistant disease was underestimated in our series since we did not obtain a bone marrow smear from every patient who apparently died from infection.

Our study, also comparing DAT and TAD sequences during postinduction therapy, shows no difference in CR duration and survival between these sequences for CR duration or for overall survival of the patients. CR duration in our patients is longer than in most series using postinduction chemotherapy that does not result in aplasia, and this could be attributed to the sequential use in our study of early and relatively intensive consolidation (17–29), maintenance chemotherapy (25,29), and final intensification (17,23), all strategies which have been associated with increased CR duration.

TABLE 1.—Characteristics of patients randomized in DAT and TAD arms

Characteristic	Arm DAT ($n = 46$)	Arm TAD ($n = 51$)
Mean age in yrs ± SD	48 ± 15	49 ± 17
Mean wbc count ± SD ($\times 10^7$ cells/L)	57 ± 106	32 ± 64
No. of patients		
Sex (male/female)	25/21	25/26
FAB type of AML		
M1	9	19
M2	7	11
M3	5	1
M4	11	9
M5	10	6
M6	2	3
Unclassified	2	2

TABLE 2.—Response to DAT and TAD arms

	Arm ($n = 46$)	Arm ($n = 51$)
Median days with neutrophil count $< 0.5 \times 10^7$/cells/L (range)	20 (11–35)	20 (14–50)
Median days with platelet count $< 50 \times 10^7$/cells/L (range)	21 (10–30)	20 (7–50)
No. of patients with (%)		
Resistant disease	1 (2)	2 (4)
Other failures	8 (17)	8 (20)
CR	37 (81)	41 (80)
Median No. of days to CR (range)	30 (21–50)	33 (21–121)*
Median No. of days in CR (range)	549 (28–1894)	518 (5–2123)
Median No. of days of survival (range)	544 (1–1929)	565 (4–2147)

*Includes 3 patients achieving CR after 2 courses.

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Phase II Trial Evaluating Continuous Infusion of Etoposide, Cisplatin, and Hexamethylmelamine in Extensive-Disease Small Cell Carcinoma of the Lung[1]

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A total of 27 untreated and 24 previously treated patients with extensive-disease small cell lung cancer (SCLC) were treated with a combination chemotherapeutic regimen of continuous-infusion etoposide for 5 days, cisplatin, and hexamethylmelamine. Of 25 evaluable patients with untreated SCLC, three (12%) achieved a complete response and 16 (64%) achieved a partial response. Among 23 evaluable patients with relapsed SCLC there were no complete responses and nine (39%) achieved a partial response. Median survival times were 252 and 109 days for the above groups, respectively. Myelotoxicity, especially thrombocytopenia, was moderately severe. Other toxic effects, including renal and neurologic, were minimal. These results compare favorably with other regimens including etoposide and cisplatin. Our results further confirm the activity of etoposide and cisplatin as both initial therapy and as a salvage regimen in the management of patients with extensive-stage SCLC. [Cancer Treat Rep 71:575-580, 1987]

Approximately 25% of lung cancer patients will have small cell lung cancer (SCLC) of which one-third will have limited disease and two-thirds will have extensive disease. It has long been recognized that in the treatment of SCLC the attainment of a complete response (CR) is tantamount to prolonged survival, and a small proportion of patients achieving CR will be cured (1-8). Towards this end, a multitude of different combination chemotherapeutic regimens have been reported in the hopes of increasing the complete response rate and thus prolonging survival. We report on a new combination chemotherapeutic regimen including continuous infusion of etoposide, cisplatin, and hexamethylmelamine in the management of SCLC patients with extensive disease, both untreated and previously treated. The rationale for this regimen is briefly summarized below.

The semisynthetic podophyllotoxin derivative etoposide is known to be one of the most active single agents in SCLC, achieving objective responses varying between 20% and 65% (1,7,9-16,20). Recent work into the mechanism of action of etoposide suggests it may induce DNA strand breaks by inhibition of topoisomerase II (17, 19,42). Pharmacologically, etoposide has a biexponential decay curve with a terminal half-life of approximately 11 hours (14,19). It is also recognized that resistance may be associated with diminished steady-state drug levels (19). Considerable schedule dependency has been observed, and it is known that the effectiveness of etoposide is greatest when it is administered in a repetitive dosage schedule as opposed to a single injection (12,13, 18-20). The maximal tolerated dose of etoposide by a continuous 5-day infusion has been described (18). Thus, it is hoped that the maximal drug level and response to etoposide can be achieved by a continuous infusion of the drug.

Cisplatin is not thought to be an active single agent in SCLC. On average, its response rate varies between 0% and 20% (21-25). The drug, however, is known to be synergistic with etoposide in animal tumor models (17,43,44). This has prompted many investigators (29-40,45) to use this combination in humans.

Hexamethylmelamine is active in SCLC (1,4,7,25-28) and has been used in combination with etoposide (4,25). The attractiveness of this agent includes its minimal myelotoxicity and the fact that it is administered orally. It is used in combination with etoposide and cisplatin in

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the hopes of providing an active agent towards the end of the chemotherapeutic cycle, during the period of bone marrow recovery.

MATERIALS AND METHODS

Patients with SCLC were entered onto one of two protocols, untreated and previously treated, between February 1982 and February 1985. Participating institutions included: University of Wisconsin Hospital and Clinics, William S. Middleton Veterans Administration Hospital, Gundersen Clinic, Marshfield Clinic, and the University of Rochester, New York. All patients: signed informed consent; had histologically confirmed SCLC by light microscopy or with an unequivocally positive cytologic diagnosis (sputum, at least 2, or aspiration biopsy) only were admitted; had extensive disease, that is, disease involving contralateral supraclavicular lymph nodes, contralateral hemithorax, pleural effusion, or any evidence of distant metastatic spread; had measurable or evaluable disease; Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 3; were < 75 years of age; were free of active infection prior to entry onto the study. Previously treated patients had had no chemotherapy for 3 weeks prior to entry onto study (6 weeks for a nitrosourea); and finally, all patients had to have adequate bone marrow (wbc count $\geq 4000/mm^3$ and platelet count $\geq 100,000/mm^3$), liver (bilirubin ≤ 2.0 mg/dl), and renal (creatinine ≤ 1.5 mg/dl and BUN $\leq$ 25 mg/dl) functions. History and physical examination, tumor measurements (product of greatest perpendicular diameters of indicator lesions), cbc and platelet count, serum creatinine, serum chemistries and electrolytes, and chest x-ray were performed prior to initiation of therapy and prior to each succeeding 3-week cycle of treatment. Audiometry, bone scintigraphy, and electrocardiograms were performed prior to initiation of therapy and as clinically indicated thereafter. Bone marrow aspiration and biopsy, cranial computed tomography, and liver scintigraphy were considered optional unless the patient was symptomatic.

TREATMENT REGIMEN

A variety of antiemetic regimens were prescribed during the course of this trial. The dose of etoposide was 50 mg/m^2 given as a continuous infusion via a central line on Days 1 through 5. The computed daily dose was divided into four equal 6-hour infusions in 500 ml of normal saline. An infusion pump was employed and the solution protected from light exposure by aluminum foil. Iv hydration was begun at the conclusion of the etoposide infusion with 5% dextrose in one-half normal saline plus 10 mEq/L KCl at a rate of 150 ml/hour for 12 hours. Cisplatin was administered at a dose of 75 mg/m^2 given as a bolus injection at the end of 12 hours' hydration on Day 6. Prior to this infusion, a forced diuresis was obtained by administration of 40 mg of furosemide followed by 12.5 g of mannitol given as an iv push. Hexamethylmelamine was administered at 150 mg/m^2 orally in a split dose twice daily, preferably after meals on Days 8 through 17. The above regimen was repeated every 21 days. All patients received at least one cycle of chemotherapy.

For untreated patients who obtained a CR, prophylactic cranial irradiation was administered as 3000 rads midplane tumor dose. This was delivered over 10 days and after induction, which was considered after completion of three cycles of chemotherapy. The subsequent cycle of chemotherapy was delayed for the 2 weeks required to complete irradiation.

Dosage modifications for subsequent cycles of chemotherapy were based on nadir and treatment day blood counts as follows. If wbc count was $\leq 3000/mm^3$ or platelet count was $\leq 75,000/mm^3$ on Day 1 of a new cycle, treatment was postponed until wbc count was $\geq 3000/mm^3$ and platelet count was $\geq 75,000/mm^3$. At that time, if the wbc count was $\geq 4000/mm^3$ and platelet count was $\geq 100,000/mm^3$ a full dosage of drugs were administered. If wbc count was between 3000 and $3999/mm^3$ or platelet count was between 75,000 and $99,000/mm^3$, a 25% reduction was made in the dose of etoposide only. For a wbc count between 2000 and $2999/mm^3$ or platelet count between 50,000 and $75,000/mm^3$, etoposide and cisplatin were held and hexamethylmelamine was administered at full dosage. Treatment was withheld entirely for wbc count $< 2000/mm^3$ or platelet count $< 50,000/mm^3$. If nadir wbc counts of the preceding cycle were $< 1000/mm^3$ or nadir platelet counts were $< 30,000/mm^3$, a 50% dosage reduction was made for all three drugs. Subsequently, if nadir counts improved from above determinations, the dose of all drugs was returned to 75% of the initial dose. Cisplatin dosage was reduced 50% for serum creatinine between 1.5 and 2.5 mg/dl and withheld for serum creatinine ≥ 2.5 mg/dl. A 50% reduction in etoposide and hexamethylmelamine dosage was made for serum creatinine ≥ 3.5 mg/dl. Dose modifications were made for etoposide only for severe hepatic dysfunction. When bilirubin was between 3.1 and 5.0 mg/dl, a 50% reduction was made, and when bilirubin was > 5.0 mg/dl, a 75% reduction was made. Severe and incapacitating neuropathy prompted withholding of hexamethylmelamine first and if it progressed, cisplatin was then discontinued.

Patients were evaluated for response at the conclusion of each cycle of therapy according to standard ECOG criteria (41). At the completion of six cycles of chemotherapy, all CRs were to be restaged with bronchoscopy, computed tomography, and other appropriate studies which were initially abnormal. If a CR was pathologically confirmed, patients were followed without maintenance therapy. Partial responders (PRs) remained on protocol until two sequential cycles of therapy resulted in stable disease. Thereafter, they were treated with a non-cross-resistant regimen of cyclophosphamide, doxorubicin, and vincristine (CAV). Patients with stable disease after completing three cycles of chemotherapy were taken off protocol and treated with a CAV regimen. Patients with progressive disease (PD) manifested by

TABLE 1.—Patient characteristics*

	Untreated	Relapsed
Total	25	23
Male:female ratio	21:4	12:11
Median age (yrs)	62	55
Range of age (yrs)	36–76	44–73
Performance status (ECOG)		
0	3	2
1	17	11
2	4	9
3	1	1

*Unless otherwise indicated, values = No. of patients.

intracranial metastases were continued on chemotherapy with the addition of irradiation and corticosteroids for local control. Patients with PD were taken off protocol at the conclusion of at least one cycle of chemotherapy and were to be included in all subsequent analyses of response and survival. Finally, a primary measure of therapeutic effectiveness in this study was survival.

RESULTS

Twenty-seven patients with untreated extensive-stage SCLC were enrolled, of whom 25 were evaluable. One patient was not evaluable because of documentation of prior systemic therapy, and another was excluded because a carcinoma of the cecum was determined as the primary lesion on autopsy. Another patient was 76 years old at the time of enrollment and was considered eligible for study. Of the 25 patients (table 1), 21 were men and four were women. The median age was 62 years. Eighty percent of patients had a performance status of 0 or 1, ECOG scale.

Twenty-four patients with relapsed extensive stage SCLC were enrolled. One patient was not evaluable because of the institution of treatment before allowable by protocol. Of the 23 evaluable patients (table 1), 12 were men and 11 were women, with a median age of 55 years. Fifty-seven percent of patients had a performance status of 0 or 1, ECOG scale.

Of the 25 untreated patients, three (12%) obtained a CR and 16 (64%) obtained a PR for an overall response rate of 76% [95% confidence interval (CI), 55–90]. One patient (4%) had stable disease and five patients (20%) had PD. Median survival time (MST) for this group of patients was 252 days (36 weeks; *see* fig 1).

Four patients died early and were considered to have PD. One died on Day 8 of Cycle 1, and three died during the course of the second cycle of chemotherapy. Of these, a single autopsy was performed in a patient who succumbed from sepsis. Postmortem examination confirmed PD and a right bilobar pneumonia as a possible source of his sepsis. It was not thought that this patient died as a complication of therapy. Another patient died as a complication of therapy from pseudomonal sepsis during a period of drug-induced neutropenia. Two other patients died unexpectedly.

Of the 23 relapsed patients, no one obtained a CR. Nine patients (39%) did achieve a PR. Thus, the overall response rate for this group was 39% (95% CI, 20–61). One patient (4%) had stable disease and 13 patients (57%) had PD. The MST for this group of patients was 109 days (15.5 wks; *see* fig 2).

Two of the relapsed patients died early during the course of the first cycle of chemotherapy. Of these, a single autopsy was performed and confirmed PD. The other patient was considered to have PD. All six of the patients with early death were included in response and survival analyses.

Eleven of the 25 untreated patients (44%) did not receive additional therapy after failing etoposide, cisplatin, and hexamethylmelamine. Seven patients died and four patients refused additional therapy. Of the remaining 14 patients (56%), four were treated with combination chemotherapy and some form of radiotherapy (either intracranial or primary lung irradiation) and ten received chemotherapy alone. The majority of these subsequently treated patients (ten of 14, 71%) received CAV as part of their chemotherapeutic regimen. The median number of months these patients were treated with chemotherapy was 1.5 (range, 1–7).

The ten patients with relapsed SCLC were treated with a CAV regimen for a median of 1.5 months (range, 1–4). No responses were observed. All patients had PD and died shortly after discontinuation of therapy.

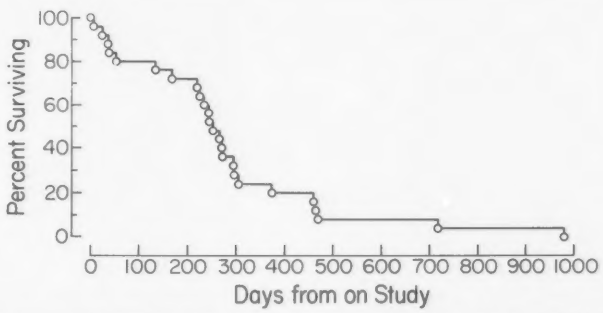

FIGURE 1.—Plot of survival in 25 untreated patients with extensive-stage SCLC. MST was 252 days (36 wks).

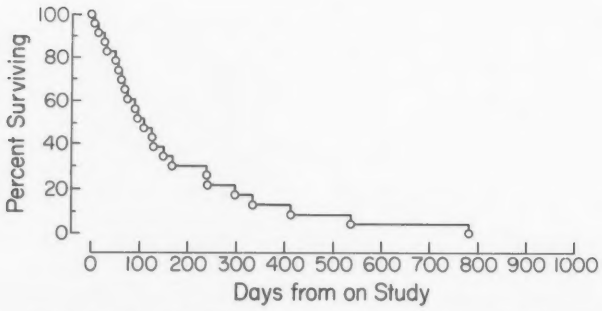

FIGURE 2.—Plot of survival in 23 relapsed patients with extensive-stage SCLC. MST was 109 days (15.5 wks).

Of the 23 previously treated patients, 14 (61%) were initially treated with combined modality treatment and nine (39%) were treated with chemotherapy alone. Again, the majority of patients (18 of 23, 78%) were treated with a CAV regimen as part of their initial chemotherapy. The median number of months these patients were treated was 3.5 (range, 1–17). The median interval between completion of primary therapy and subsequent initiation of treatment with etoposide, cisplatin, and hexamethylmelamine was 1 month (range, 1–9.5).

TOXICITY

This regimen was for the most part tolerable. Myelotoxicity was most pronounced and thrombocytopenia perhaps more severe than anticipated. The toxic effects encountered are summarized in table 2.

Severe and life-threatening neutropenia occurred in 52% of untreated patients and 43% of previously treated patients. The majority of these patients had wbc counts between 1000 and 2000/mm^3 or an absolute neutrophil count of between 500 and 1000/mm^3 (grade 3 toxicity). Twelve percent of untreated patients and 4% of previously treated patients had a wbc count of < 1000/mm^3 or an absolute neutrophil count of < 500/mm^3 (grade 4 toxicity). A single patient died as a complication of therapy. This patient never recovered from pseudomonal sepsis, which was documented 2 days after a wbc count nadir of 300/mm^3 on Day 13 of the second cycle of therapy.

TABLE 2.—Summary of toxic effects by ECOG criteria*

	Untreated patients ($N = 25$) Grade					Relapsed patients ($N = 23$) Grade				
	0	1	2	3	4	0	1	2	3	4
Wbc count	2	3	7	10	3	1	4	8	9	1
Platelet count	3	3	4	6	9	2	3	7	5	6
Hematocrit	6	1	3	15	—	5	2	2	14	—
Renal	12	11	2	0	0	16	5	2	0	0
Hepatic	25	0	0	0	0	23	0	0	0	0
N + V	1	6	18	0	0	3	8	8	4	0
Neurologic	12	10	0	3	0	11	4	7	1	0
Auditory	17	8	0	0	0	23	0	0	0	0

*Grade: 0 = no toxicity; 1 = mild; 2 = moderate; 3 = severe; and 4 = life threatening. N + V = nausea and vomiting. Nos. in given column refer to No. of patients with stated toxicity. Details of toxicity grading have been outlined by ECOG (41).

Thrombocytopenia of $< 50{,}000$ cells/mm^3 occurred in 60% of untreated patients and in 48% of previously treated patients. This is further broken down as follows. Platelet counts between 25,000 and 50,000/mm^3 (grade 3 toxicity) occurred in 24% of untreated patients and in 22% of previously treated patients. Platelet counts $< 25{,}000$/mm^3 (grade 4 toxicity) occurred in 36% of untreated patients and in 26% of previously treated patients. This degree of thrombocytopenia is slightly more severe than reported by others (18,29–31,36).

Sixty percent of patients in both groups required rbc transfusions (grade 3 toxicity). In the untreated patients, median pretreatment hemoglobin levels were 13.9 g/dl (range, 10.5–17 g/dl) and median posttreatment hemoglobin levels were 11.2 g/dl (range, 7.2–15.1 g/dl). The corresponding median hemoglobin values in relapsed patients were 12.5 g/dl (range, 7.7–16.5 g/dl) pretreatment and 10.9 g/dl (range, 7.5–13.8 g/dl) posttreatment. Overall, myelotoxicity tended to be cumulative.

Other toxic manifestations were considerably less common and severe. Ninety percent of patients in both groups experienced minimal to no renal toxicity. No hepatic toxicity was encountered. Nausea and vomiting was controllable in all instances. Neurologic toxicity was disabling in 12% of untreated patients. Two patients suffered from severe peripheral sensory neuropathy of the lower extremities from which there was no recovery. A single previously treated patient experienced disabling motor weakness of the left hand. No other severe neurologic toxic effects were encountered in this group despite the fact that the majority had received vincristine in their prior treatment regimens. And finally, there was no significant auditory toxicity.

DISCUSSION

The combination regimen of continuous-infusion etoposide, cisplatin, and hexamethylmelamine is active in extensive-stage SCLC. The 76% response rate and

median survival of 36 weeks in untreated patients compares favorably with others (31–33,37–40). Sierocki and others (38) initially reported a median survival of 36 weeks in 17 patients with extensive-stage SCLC treated with an alternating cisplatin-etoposide and CAV regimen for 18 months. Natale and Wittes (39) later reported an improved median survival of 14 months in 20 extensive-stage SCLC patients. These patients received maintenance chemotherapy after completing an alternating CAV and etoposide-cisplatin induction regimen. A more recent trial by Evans and others (31) reported a median survival of 39 weeks in 17 patients with extensive-stage SCLC treated with monthly cycles of etoposide and cisplatin. The above series and our group of untreated patients were comparable in median age, sex distribution, and overall good to excellent performance status at the beginning of therapy.

In relapsed patients, the 39% response rate is also similar to that previously reported (29,30,34–36). This latter observation is important and suggests that our regimen is non-cross resistant when conventional combination chemotherapeutic regimens have failed. Caution is needed, however, as Batist and others (45) reported a very limited response rate with an etoposide and cisplatin combination in previously treated patients. Their data suggest that the induction of resistance may relate to the intensity of prior therapy.

The regimen employed in this trial was tolerable although myelotoxicity was pronounced. The degree of thrombocytopenia (60% of untreated patients had grade 3 toxicity) was greater than reported for other etoposide and cisplatin regimens. While the addition of hexamethylmelamine may account for increased myelosuppression, it is more probable that this reflected the manner in which the etoposide was administered. The continuous infusion of etoposide has previously been demonstrated to produce moderately severe thrombocytopenia (18). Other toxic effects, including renal and neurotoxicity, were tolerable and not found to be dose limiting in these patients.

In summary, this report constitutes one of the largest trials of an etoposide- and cisplatin-based combination regimen in extensive-stage SCLC. Our data confirm the activity of etoposide, cisplatin, and hexamethylmelamine combination therapy in this disease as both an induction and salvage chemotherapy regimen. The experience reported for this three-drug regimen is no better or worse than that with etoposide and cisplatin alone. Thus, it is not likely that the schedule of etoposide used in this trial or the addition of hexamethylmelamine added significantly to the observed responses. The non-cross-resistant activity of our regimen as salvage therapy suggests that etoposide and cisplatin should be tested as part of alternating combination regimens in the initial management of SCLC.

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Demonstration of a Schedule-Dependent Therapeutic Synergism Utilizing the Interacting Drugs Methotrexate and Teniposide in L1210 Leukemia[1,2]

Galen L. Wampler,* Walter H. Carter, Jr, Eleanor D. Campbell, and I. David Goldman[3,4]

When three variables require simultaneous adjustment for treatment optimization, the experimental determination of the optimum treatment becomes more complicated than generally appreciated, especially when one variable is the interval between drug administrations. Molecular pharmacological studies at this institution suggest that teniposide, in doses that are achievable in vivo, blocks methotrexate efflux from cells, enhancing formation of methotrexate polyglutamates which are active retentive forms of the drug. Hence, the combination might show a synergistic effect if teniposide is given at an appropriate time in relation to administration of methotrexate. This paper considers the problem of estimating the optimal dose levels and timing of administration of these drugs in $B6D2F_1$ mice bearing L1210 leukemia in vivo as a model for the analysis of multidrug regimens when time and dose are variables. Because of the complexity of these experiments, an adaptive approach was applied. Three cycles of treatment were given using methotrexate at 0–400 mg/kg, teniposide at 0–60 mg/kg, and an interval of 0–54 hours between the two drugs. The single drugs prolonged median survival up to 24 days under the conditions of these experiments. The combination given simultaneously resulted in median survival of up to 33.5 days, while use of an appropriate interval between drugs prolonged median survival to > 50 days. An analysis of the underlying dose-time response gives a much better appreciation of the relationships among the variables. The concept that optimal doses included less methotrexate and more teniposide as the interval between drugs is increased was developed only through modeling. This finding is critical in demonstrating the importance of including all nonnegligible variables in the experimental design if the results are to be considered valid. Confidence regions about the optimum dose and about the response at the optimum provide a sound basis for a claim of therapeutic synergism as demonstrated by use of a scheduling variable in the experiment design. A nonproportional hazards analysis permits the conclusion of nonproportionality and emphasizes the contribution of methotrexate to optimal short-term survival (15–24 days) and teniposide to long-term survival (24–39 days). [Cancer Treat Rep 71:581–591, 1987]

There has been a long history of interest in the effects of drug scheduling in relationship to treatment efficacy. Treatment programs have been more or less arbitrarily chosen both for animal and human experiments. Optimum schedules for single drugs in animals have been published based on the study of a limited number of the schedules that could be devised (1). A number of problems arise in studies on drug scheduling with drug combinations. One obvious difficulty is the combining of drugs with different schedule dependencies. Another problem unique to combinations is the determination of whether the drugs should be given simultaneously or separately. If it appears that separate administration results in better treatments, the question of the optimal interval between drugs is raised. It would seem desirable to have a means of defining, at a predetermined level of significance, that one way of administering a combination of drugs is better than another or, better still, to be able to establish that a particular regimen is the optimal way that a combination of drugs can be

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utilized. This paper considers the problem of estimating the optimal dose levels and interval between administrations of the drugs in a two-drug combination, using response surface methodology (2–4).

Yalowich et al (5) studied the effects of teniposide (VM-26) and etoposide on methotrexate (MTX) transport and polyglutamylation in Ehrlich ascites tumor cells in vitro. In this system the epipodophyllotoxins slowed MTX efflux, resulting in enhanced net cell accumulation of MTX and an increase in MTX polyglutamyl derivatives. VM-26 was ten times more active in this respect than etoposide. These findings raised the possibility that if adequate levels of the epipodophyllotoxins were given at an appropriate time, augmentation of the antitumor effect of MTX might be achieved. Because of a well-defined biochemical rationale for a potential synergistic interaction between these agents, we selected the combination of MTX and VM-26 for a study of the effect of interval between drug administrations on therapeutic efficacy in the L1210 tumor system in vivo.

MATERIALS AND METHODS

Tumor System, Animals, and Drugs

L1210 leukemia, maintained in DBA/2 mice, was passed weekly using 10^5 cells ip. B6D2F$_1$ (C57BL/6 × DBA/2) female mice weighing 16–20 g were used as test animals. Each received 1×10^5 L1210 cells ip. Mice were kept in an air-conditioned, light-controlled environment in groups of two or four per cage and were given standard laboratory formula and water ad libitum. MTX, supplied by the National Cancer Institute (NCI), was dissolved using NaOH, then buffered with $NaHCO_3$. The VM-26 clinical preparation, supplied by NCI, was diluted in saline for use. Treatment was started on Day 3 after tumor inoculation and was repeated every 4 days for three cycles of treatment. A single dose of MTX every 4 days is the published optimal treatment schedule for MTX (6). This same schedule is a reasonable schedule for VM-26 (7). All drugs were given by ip injection at 10 AM or 4 PM, except for a final experiment where MTX was given at 10 AM and VM-26 was given 20, 25, 30, 35, or 40 hours later. Animals were observed daily for deaths.

Experimental Design

An adaptive approach was utilized. The initial experiment covered rather sparsely a wide range of doses and intervals. After analysis, a second experiment was conducted in a treatment space of interest which was contained in the 95% confidence region about the estimated optimum treatment (dose, time).

First Replication

A modified factorial design (3) for three variables was utilized: dose of MTX (six doses from 12.5 to 400 mg/kg; logarithmic scale), dose of VM-26 (six doses from 10 to 60 mg/kg; arithmetic scale), and interval between MTX and VM-26 (0, 6, 18, 30, 42, and 54 hours). This replication used 82 groups of two animals each plus a control group of eight animals. Figure 1 shows graphically where the treatment points were located. Two animals per group were used, since we had previously shown through the use of computer simulations that, given a fixed number of animals, it is more desirable to have a small number of animals in many groups than a larger number of animals in fewer groups (3).

Second Replication

A factorial design was again used in a more restricted treatment space based on the 95% confidence region about the optimal treatment generated from an analysis of the results from the first replication. Seventy-seven groups of two animals each (one animal lost) plus eight controls were used. Treatment points were located as indicated in figure 1.

Third Replication

Treatment points (also shown in fig 1) were located within or near the 95% confidence region generated by analysis of the first two experiments combined. Sixty-two groups of two animals each plus eight controls were used.

In a final, abbreviated experiment, groups of four mice each were given fixed dose ratios of MTX and VM-26 (35:13) at 20-, 25-, 30-, 35-, or 40-hour intervals. MTX was given at 10 AM on Days 3, 7, and 11 following inoculation of 10^5 L1210 cells ip, and VM-26 was given at 6 AM, 11 AM, 4 PM, or 9 PM on Days 4, 8, and 12 or at 2 AM on Days 5, 9, and 13 for the respective groups.

The combined experiment consisted of 529 animals in 236 treatment groups, with 28 animals in the untreated control groups. Doses of MTX were increased logarithmically because MTX gives a flat dose-response curve. Hence, a dose of 25 mg/kg (lowest dose used alone) gave a median survival of 12.5 days, while 197 mg/kg gave a median survival of 13 days (control, 8 days). The logarithmic scale for MTX appears to be the most effective way to cover the range of doses that might prove to be optimal with combination treatments.

Analysis

Response surfaces estimating the relationship between survival time or, more specifically, the instantaneous risk of failure (hazard) and treatment levels, ie, dose levels of the two drugs and the interval between MTX and VM-26, were constructed through use of Cox's proportional hazards model (9).

The model used was:

$$\ln\left(\frac{\lambda(t)}{\lambda_0(t)}\right) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \gamma_1 z_1 + \gamma_2 z_2 + \gamma_3 z_3,$$

where $\frac{\lambda(t)}{\lambda_0(t)}$ was the ratio of the risk of failure (death)

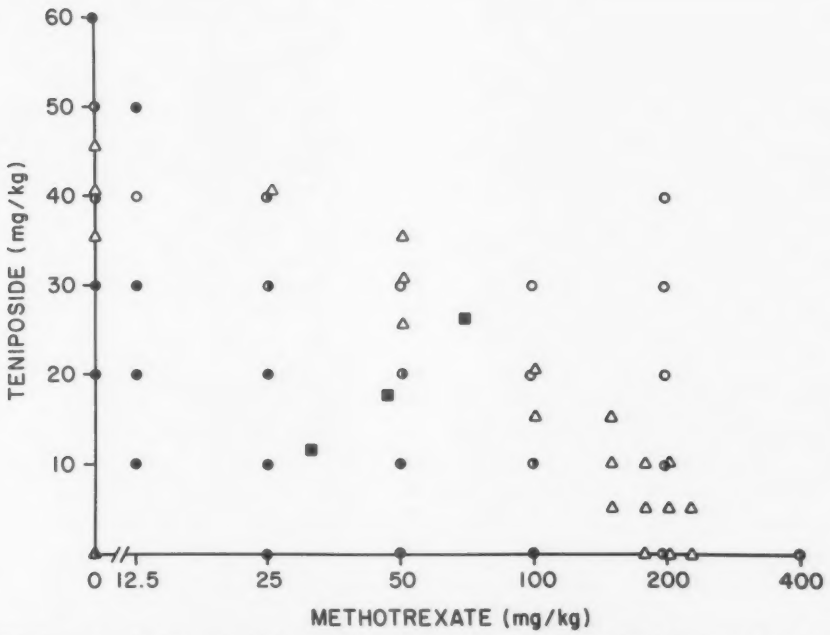

FIGURE 1.—Location of experimental treatment points. ● = first replication; all indicated treatments given at 0-, 6-, 18-, 30-, 42-, and 54-hr intervals. ○ = second replication; all treatments given at 0-, 6-, 18-, 30-, 42-, and 54-hr intervals. ◑ = both of above. △ = third replication; treatments given at 0-, 18-, and 42-hr intervals; VM-26 alone given at 18, 30, 42, and 54 hrs after time 0. ■ = final experiment; all treatments given at 20-, 25-, 30-, 35-, and 40-hr intervals.

$\lambda(t)$ at time t for the treated group to the risk of failure $\lambda_0(t)$ for the untreated control group at the same instant in time; x_1 = dose (mg/kg) of MTX [ln(MTX + 1) was used for the final analysis]; x_2 = dose (mg/kg) of VM-26; x_3 = time (hours) between MTX and VM-26; $z_1 = 1$ if replication = Replication 1, 0 otherwise; $z_2 = 1$ if replication = Replication 2, 0 otherwise; and $z_3 = 1$ if replication = Replication 3, 0 otherwise. z_1 was used for two replications; z_1 and z_2 were used for three replications; and z_1, z_2, and z_3 were used for four replications.

The β's are unknown constants which parameterize the dose-response relationship. The β's with single subscripts are related to the effects of the individual design variables (eg, β_1 is associated with effect of MTX); those with two different subscripts are associated with interactions between two of the variables (eg, β_{12} is associated with the interaction between MTX and VM-26); and those with two equal subscripts are associated with the toxic effect of a treatment variable (eg, β_{11} is associated with the toxicity of MTX). Since the experiment was done sequentially it is possible that there are extraneous uncontrolled sources of variation, ie, all observations were not taken under equivalent conditions. To account for this, three variables (z_1, z_2, and z_3) were introduced into the model. The γ's are unknown constants associated with such variables. As a result of such a parameterization of the model, the estimates of the dose-response parameters are adjusted for the variation from replication to replication. All model parameters were estimated by the method of maximum likelihood.

Optimum treatments were estimated using the method of Nelder and Mead (9). Confidence regions about the location of the optimum treatments were constructed using the method of Carter et al (4). In addition, confidence intervals about the relative hazard at the optimum treatment for various time delays were calculated by the method of Carter et al (10).

Initially the analysis was conducted using arithmetic units both for drug doses and for time. Our experience has been that using arithmetic doses as opposed to logarithmic doses gives as good or better fit to the data and obviates the requirement for working with a transformed dosage scale, since the ln of 0 is undefined. In the final analysis, the ln(MTX + 1) term was used in the model. This departure from our standard procedure was justified by the improved fit to the data obtained with the analysis. Replication effect terms were included in the model when data were combined from more than one experiment to adjust for the variation from replication to replication. Possible sources of such variation include variation in age or weight of the animals and differences in the tumor cell inoculum or drug preparation. Goodness of fit of the proportional hazard model was assessed by using Kay's method of plotting residuals (11).

The assumption of proportional hazards was examined

TABLE 1.—Summary of results before modeling: median survival times (2 animals/group)

	Experiment (Replication)		
	1	2	3
Control			
Survival (days)	8.0	7.5	9.5
Best MTX			
Survival (days)	15.5	11.0	21.0
MTX dose (mg/kg)	394	200	178
Best VM-26			
Survival (days)	18.0	18.0	24
VM-26 dose (mg/kg)	20	40	36
Best combination (drugs given together)			
Survival (days)	22.5	20.5	33.5
MTX dose (mg/kg)	199	100	178
VM-26 dose (mg/kg)	10	10	10
Best combination (interval between drugs)			
Survival (days)	28.0	21.0	40+
MTX dose (mg/kg)	25	100	25, 51
VM-26 dose (mg/kg)	40	10	40, 36
Interval between MTX and VM-26 (hrs)	54	6	42

by repeating the analysis with the addition of terms to account for interactions between treatment by time and treatment by time squared.

RESULTS

Raw data for the 236 treatment groups and 28 control animals are shown in the Appendix. Tables 1 and 2 summarize these data in a conventional manner. The classical method for determining therapeutic synergism is simply to compare single drug activity with combination drug activity to see which gives best results. In each replication, the results of the combination with an interval between the two drugs were superior to the results when the drugs were given simultaneously, and combination treatments were better than optimal doses of single drugs (table 1). In the final experiment more animals per group were given combination doses in a favorable region of the treatment space and followed for a longer period of time (table 2). Animals treated with the single drugs MTX or VM-26 did not live beyond 30 days. There were 66 animals treated with single drugs in the overall data; 20 received MTX alone at various doses and 46 received VM-26 alone at various doses and times. All died before Day 30 (data in the Appendix). Thus, the data indicate therapeutic synergism between the drugs when the interval is appropriate, using a classic definition of the term therapeutic synergism (12). However, the level of significance associated with this claim cannot be defined.

After each replication, the response surface generated was examined for optimum treatments for single drugs alone and for combinations at each of a number of intervals. These invariably showed that optimum doses at a 0 interval were nearest and not significantly different from the optimum dose of MTX alone, but as the interval increased, less MTX and more VM-26 were required for optimum conditions. The estimated hazard decreased for 31–43 hours, then increased. The size of the 95% confidence region became smaller as more data were added from additional replications. Table 3 lists Spearman's rank order correlation relating the observed survival time for each group to the estimated hazard. This provides an indication of the strength of the relationship between the model and the data. Also shown is the *P* value associated with each correlation.

TABLE 2.—Final experiment using a fixed dose ratio of MTX and VM-26 (4 animals/group)

MTX dose (mg/kg)	VM-26 dose (mg/kg)	Interval between drugs (hrs)	Median survival (days)	No. of survivors			
				30-day	50-day*	70-day*	90-day*
0	0	—	9	0	0	0	0
31.2	11.7	20	77	3	3	2	0
46.8	17.5	20	43.5	4	1	1	0
70.2	26.3	20	19.5	0	0	0	0
31.2	11.7	25	46.5	2	2	1	0
46.8	17.5	25	88	4	4	3	2
70.2	26.3	25	14	0	0	0	0
31.2	11.7	30	66	4	4	1	0
46.8	17.5	30	36	2	1	0	0
70.2	26.3	30	9	0	0	0	0
31.2	11.7	35	72	3	2	2	2
46.8	17.5	35	90	3	3	3	2
70.2	26.3	35	9	0	0	0	0
31.2	11.7	40	44	4	1	1	1
46.8	17.5	40	15	1	1	1	1
70.2	26.3	40	10.5	0	0	0	0
				30	22	15	8

*Gross autopsies performed on most of the animals dying after Day 50 showed the cause of death to be delayed peritoneal toxicity from VM-26 as described by Avery et al (7). There was no evidence of residual tumor. All but 1 of the 90-day survivors eventually died by Day 150.

TABLE 3.—Evaluation of strength of relationship between observed survival and estimated hazard after each of various replications

Replication	Spearman's rank correlation	*P* value
1	−0.7471	0.0001
1-2	−0.2735	0.0004
1-3	−0.3944	0.0001
1-4	−0.3767	0.0001
1-4*	−0.5437	0.0001

*Using ln(MTX + 1) in the analysis.

For the combined data set, the parameters of the model and the significance of each term are indicated in table 4. Parameters β_1 and β_{11} are associated with the dose of MTX; β_2 and β_{22} are associated with the dose of VM-26; and β_3 and β_{33} are associated with the time between MTX and VM-26 administration. Parameters β_{12}, β_{13}, and β_{23} are interaction terms and the γ parameters are used to account for the variability among replications. The estimated optimum doses at 6-hour intervals are plotted in figure 2 along with the ln relative hazard value associated with each point and the overall estimated optimum treatment. This result is MTX at 8.7 mg/kg followed in 42.7 hours by VM-26 at 31.3 mg/kg on the set schedule of three cycles of treatment at 4-day intervals. The 95% confidence region about the optimum treatment is a three-dimensional figure which has been plotted in figure 3 by considering the dose levels within the confidence region at 4-hour intervals. The size of the confidence region decreases up to 24 hours, then increases. It should be noted that the use of a logarithmic scale for MTX dosage tends to decrease the apparent size at higher MTX doses and increase it for lower doses.

The 95% confidence intervals about the ln relative hazard for the optimal combinations at 4-hour intervals are given in table 5. These show all negative values compared to optimum doses of single drugs from 4 to 28 hours, indicating a triple-variable therapeutic synergism for this period of time. Figure 4 is a plot of the cumulative hazard function for the crude residuals associated with this experiment. Kay (11) has suggested that the assumption of proportionality is reasonable if this relationship is described by a line of unit slope through the origin. Since there is some deviation from such a relationship, we used the method described by Stablein et al (13) and repeated the analysis with the addition of terms for treatment by time interactions. This resulted in the parameter estimates shown in table 6. Two terms for time by treatment interactions are statistically significant and the entire set of six parameters is highly statistically significant, indicating that significant nonproportionality exists. When nonproportionality is present, it is impossible to have an optimum treatment overall; rather, optimal treatments for given days of survival can be estimated. Optimum dose predictions for 15-, 24-, and 36-day survival by interval are shown in figure 5. Optimizing for 15-day survival requires lower doses of MTX than for 24-day survival and lower doses of VM-26 than for 36-day survival. Optimizing for 24-day survival requires higher doses of MTX and lower doses of VM-26 than for 36-day survival. But in each case, as interval is increased, more VM-26 and less MTX were required for optimum treatment.

In figure 6 the estimated optima for an interval of 24 hours are shown for 15–39-day survival in 3-day increments from the nonproportional hazards analysis. Other plots (not shown) for various intervals show the same relationship. To optimize for survival from 15 to 24 days requires progressively higher doses of MTX. For survival times > 24 days, lower doses of MTX and higher doses of VM-26 are required.

DISCUSSION

The rationale for the evaluation of possible therapeutic synergism between MTX and an epipodophyllotoxin is based upon the biochemical interaction between these agents. Epipodophyllotoxins block MTX efflux and augment polyglutamylation of MTX to active derivatives which are considered now to be key determinates of the cytotoxicity and selectivity of this agent (14). However,

TABLE 4.—Parameters of the combined proportional hazards model with statistical evaluation of terms

Parameter	Estimate	Standard error	Chi-square	*P* value
β_1 ln(MTX + 1)	−1.501	0.137	120.72	< 0.0001
β_2 VM-26	−0.117	0.0170	47.26	< 0.0001
β_3 interval	−0.0116	0.0145	0.63	0.4259
β_{11}	0.176	0.0214	67.67	< 0.0001
β_{22}	1.78×10^{-3}	2.97×10^{-4}	35.72	< 0.0001
β_{33}	4.65×10^{-4}	1.76×10^{-4}	6.97	0.0083
β_{12}	0.0209	2.50×10^{-3}	69.57	< 0.0001
β_{13}	1.11×10^{-3}	2.06×10^{-3}	0.29	0.5886
β_{23}	-9.80×10^{-4}	2.36×10^{-4}	17.30	< 0.0001
γ_1	0.937	0.131	50.80	< 0.0001
γ_2	−0.556	0.136	16.81	< 0.0001
γ_3	−0.941	0.204	21.17	< 0.0001

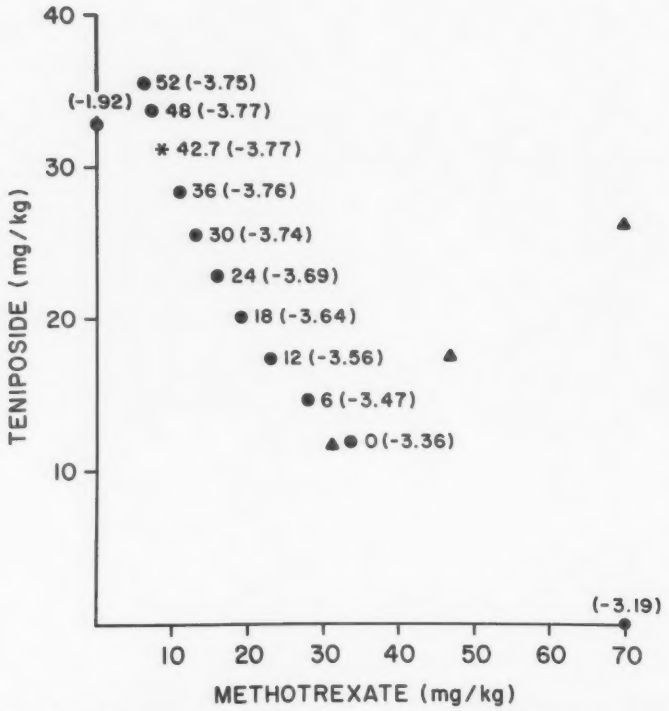

FIGURE 2.—Estimated optimum treatments at varying intervals of time between drug administration for the proportional hazards analysis. The estimated ln relative hazard associated with each point is shown in parentheses. Single-drug optima with associated estimated ln relative hazard are also shown. The time between MTX and VM-26 administration is given in hrs. Asterisk indicates overall estimated optimum treatment. Triangles indicate experimental points for which data are shown in table 2.

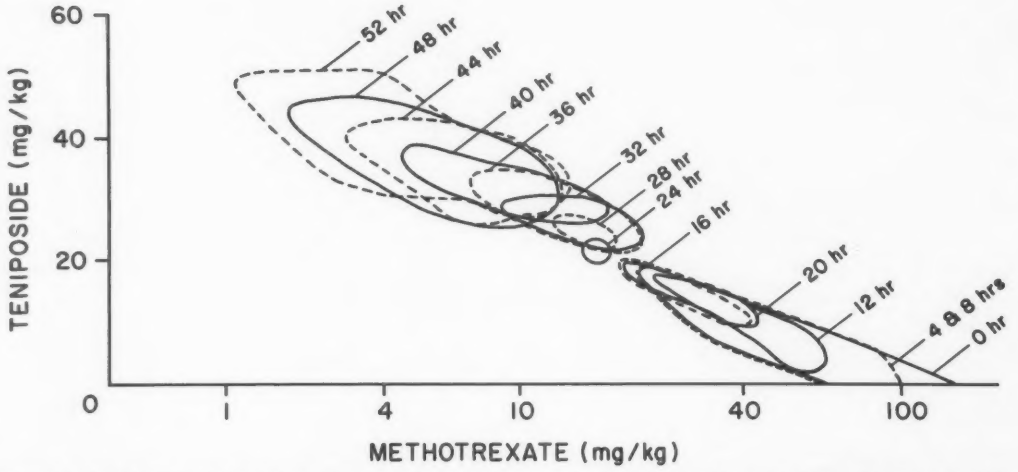

FIGURE 3.—Confidence region (95%) about the estimated optimum treatment for the proportional hazards analysis. This region should be thought of as a 3-dimensional figure, where the depth dimension has been sliced at 4-hr intervals and presented as overlays on the 2-dimensional presentation. Note the logarithmic scaling of the MTX dose.

TABLE 5.—95% confidence intervals for response at optimum combinations compared to optimum single-drug treatment*

Interval between drugs (hrs)	95% confidence intervals vs optimum MTX†§	vs optimum VM-26‡§	Evaluation¶
0	−0.4305 to 0.0558	−1.7085 to −1.2222	
4	−0.5057 to −0.0165	−1.7837 to −1.2945	TS
8	−0.5727 to −0.0713	−1.8507 to −1.3493	TS
12	−0.6496 to −0.0987	−1.9276 to −1.3767	TS
16	−0.7580 to −0.0995	−2.0360 to −1.3775	TS
20	−0.8689 to −0.0889	−2.1469 to −1.3669	TS
24	−0.9797 to −0.0669	−2.2577 to −1.3449	TS
28	−1.0902 to −0.0279	−2.3681 to −1.3058	TS
32	−1.1973 to 0.0229	−2.4752 to −1.2551	
36	−1.3047 to 0.0846	−2.5827 to −1.1933	
40	−1.4162 to 0.1629	−2.6942 to −1.1151	
44	−1.5329 to 0.2515	−2.8109 to −1.0265	
48	−1.6496 to 0.3505	−2.9276 to −0.9275	
52	−1.7663 to 0.4598	−3.0443 to −0.8182	

*Response given is ln relative hazard.

†$\ln\left[\frac{\lambda \text{ combination (t)}}{\lambda \text{ MTX (t)}}\right]$

‡$\ln\left[\frac{\lambda \text{ combination (t)}}{\lambda \text{ VM-26 (t)}}\right]$

§Negative values for the ratio indicate that the risk at the optimal combination is less than the risk associated with the optimal single drug. When the upper bound and the lower bound are both negative for each drug there is an indication that the result of treatment with the optimal combination is better than that associated with optimal single-drug treatments and thus therapeutic synergism can be claimed.

¶Therapeutic synergism (TS) is demonstrated at these time intervals.

extrapolating these findings to in vivo systems is complicated by a variety of factors, among which are binding of epipodophyllotoxins to plasma proteins and the different effects of these agents on tumor and host cell kinetics. Nonetheless, these studies indicate that there is therapeutic synergism when VM-26 is administered after MTX. Whether this synergism is based upon the known biochemical interaction between these drugs or some other mechanism is not established. The interaction is similar to that observed with MTX and vincristine, with maximal synergism demonstrated when the

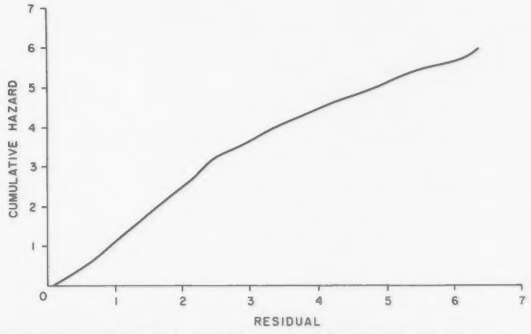

FIGURE 4.—Estimated cumulative hazard functions for the proportional hazards model residuals.

TABLE 6.—Parameters of the nonproportional model with statistical evaluation of terms

Parameter	Estimate	Standard error	Chi-square	P value
β_1 ln(MTX + 1)	−1.0976	0.2749	15.94	< 0.0001
β_2 VM-26	−0.2544	0.0363	49.12	< 0.0001
β_3 interval	−0.0042	0.0291	0.02	0.8875
β_{11}	0.1689	0.0221	58.25	< 0.0001
β_{22}	0.0021	0.0003	43.30	< 0.0001
β_{33}	0.0006	0.0002	10.18	0.0014
β_{12}	0.0217	0.0027	66.75	< 0.0001
β_{13}	0.0024	0.0023	1.14	0.2857
β_{22}	−0.0012	0.0003	19.56	< 0.0001
γ_1	0.8940	0.1343	44.29	< 0.0001
γ_2	−0.5498	0.1386	15.74	< 0.0001
γ_3	−1.0306	0.2202	21.91	< 0.0001
δ_1 MTX × time	−0.0251	0.0310	0.65	0.4201
δ_2 VM-26 × time	0.0155	0.0041	14.16	0.0002
δ_3 interval × time	−0.0019	0.0026	0.55	0.4583
δ_{11} MTX × (time^2)	0.0000	0.0010	0.0004	0.9840
δ_{22} VM-26 × (time^2)	−0.0004	0.0001	12.75	0.0004
δ_{33} interval × (time^2)	0.0001	0.0001	0.48	0.4884
All δ combined*			29.83	< 0.0001

*Likelihood ratio test statistic for the set of six time by treatment interaction coefficients.

latter was administered ≥ 16 hours after the MTX. Again, the basis for the synergism in that study was not defined (15).

The modeling technique employed in this study provides a clearer understanding of relationships between treatment variables and indicates that as the time increases between MTX and VM-26 administration, optimum doses of MTX decrease and optimum doses of VM-26 increase. A major advantage of these procedures

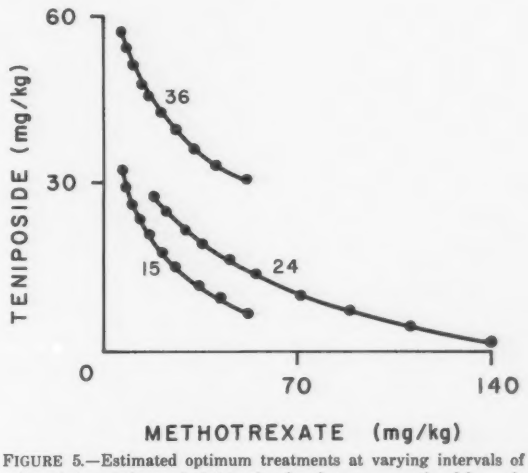

FIGURE 5.—Estimated optimum treatments at varying intervals of time between drug administration for the nonproportional hazards analysis. The three curves represent optima by interval for 15-, 24-, and 36-day survival. The 10 points on each curve represent optima for intervals of 0, 6, 12, 18, 24, 30, 36, 42, 48, and 54 hrs (from right to left).

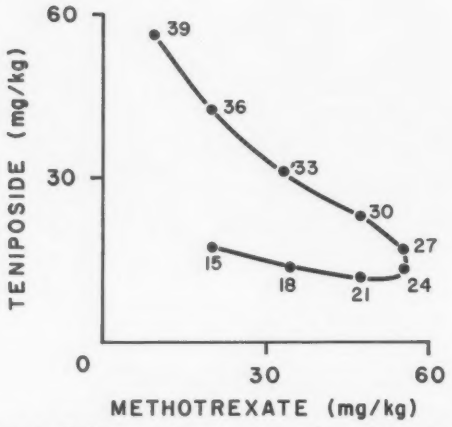

FIGURE 6.—Estimated optimum treatments for a 24-hour interval between MTX and VM-26 from the nonproportional hazards analysis for various desired lengths of survival. Points are optima for 15-, 18-, 21-, 24-, 27-, 30-, 33-, 36-, and 39-day survival (from bottom to top).

is that statistical tests have been developed to quantitate the significance of any differences detected and to define more precisely drug interactions including therapeutic synergism. In these studies significant effects were observed for each drug alone and the interval between the administration of the agents. The adaptive method utilized allows an assessment of the potential for demonstrating a result prior to the commitment of massive resources to the project. If the initial results are promising, further replications may reduce the size of the confidence regions in order that statements at a desired level of confidence can be made.

A problem was encountered with the model, which initially estimated optimum doses that were in the toxic range. This is demonstrated by the abbreviated final experiment which attempted to show that 26 mg/kg of VM-26 35 hours after 70 mg/kg of MTX was an optimal treatment. This dose ratio at four-ninths and two-thirds of the suggested optimum dose was a superior treatment, giving 50% 90-day survivors (table 2). In an attempt to rectify this discrepancy, it was noted that properties of the model result in symmetric response contours resulting in distortion of the data. This problem had been recognized but had not previously presented any major difficulty in application (2). In the present application, optima estimated for different intervals between drugs fall on an axis of a symmetrical response contour that is a straight line. In this case it was obvious that this property of the model produced an important distortion. Because of this problem the data were reanalyzed using ln(MTX + 1), which allowed a curvilinear relationship. This resulted in better fit of the model to the experimental data and an optimum dose-time estimation that was not in a toxic range. Since the models used are totally empiric, it now seems desirable to identify approaches that will increase the flexibility of the model without introducing additional terms. One such approach is the use of inverse polynomials which have no built-in symmetry (16).

The nonproportional hazards analysis is instructive, in that MTX seems to be the important drug for optimization of survival times up to 24 days. At all intervals, progressively higher doses of MTX are required for optimum treatment up to this point. For survival times > 24 days, VM-26 seems to be the important drug, with the requirement of progressively higher doses along with lower doses of MTX for optimum treatment. Although significant nonproportionality exists, as indicated by the P value of < 0.0001 for the combined time by treatment interaction terms in the nonproportional model (table 6), it is not likely that the practical impact of this statistical improvement is important. In a comparison of the estimated survival distributions from proportional and nonproportional models, Stablein et al (13) found little difference. The nonproportional analysis appears to be most useful in further defining the interactions between the drugs. Thus, we are able to conclude that in this treatment setting MTX is relatively more important with short intervals and for shorter survival optimizations, while VM-26 is relatively more important with longer intervals and for long-term survival optimization.

There are a number of experimental problems unique to studies involving intervals between drug administrations. In comparing the interval results to the results of simultaneous drug administration, if treatment were started at the same time after tumor inoculation, those groups receiving simultaneous treatment complete their regimen earlier. This generally should imply a more favorable prognosis when animals live to die of tumor rather than toxicity. Additionally, there will be circadian rhythm variations in time of treatment between the groups compared, and these have been shown to influence results in certain situations (17–20). The simplest interval experiment that can be devised involves two variables of drug dose and one time variable. Three-variable experiments are quite complicated if performed properly, as evidenced by the design for a three drug dose variable experiment proposed by Goldin et al (21). Interval is more complicated than drug dose, since drugs can be sequenced two ways and because a more complicated interplay of factors influences this variable, making it difficult to predict where experimental time points should be placed. As a result of drug toxicity it can be assumed that a second-order equation will fit a drug dose curve, but this may not be the case for a time interval curve where several peaks of efficacy could occur.

It is important to note that if two of the variables were optimized with this combination, following which the third variable was added, the optimum conditions could not be found. That is, if at a 24-hour interval the optimum doses of MTX and VM-26 are 15.9 mg/kg and 22.8 mg/kg, respectively (fig 2), and then the interval is varied using this dose ratio, 24 hours would be optimal. Thus, pilot studies or adaptive approaches, op-

timizing for a limited number of variables and then adding another while holding the original variables constant, will most likely fail. In fact, initial experiments using such an approach were found to be unsatisfactory. Should it be desired to include a fourth variable, the experimental design would need to allow variation in the three variables already shown to be important. This would require an adaptive approach because of the size of the experiment. As with all analytical methods the accuracy of prediction is dependent upon the importance of the effect of factors assumed to be negligible. This study demonstrates the importance of including all of the variables to be investigated in the experimental design at the outset. This requires an adaptive approach when three or more variables are considered. The adaptive approach may have clinical application.

APPENDIX

Survival times (days) of all mice by treatment: Replication 1

Groups	MTX (mg/kg)	VM-26 (mg/kg)	Survival times by interval between drugs*					
			0 hrs	6 hrs	18 hrs	30 hrs	42 hrs	54 hrs
1	0	0	8(7), 9					
2	0	19.9	17, 19					
3	0	29.9	15, 16					
4	0	39.9	13, 14					
5	0	49.9	11, 12					
6	0	59.9	11(2)					
7–12	12.4	9.9	14, 17	18, 19	19, 20	19(2)	13, 18	12, 15
13–18	12.4	19.9	17(2)	19, 20	22, 26	20, 23	13, 19	18, 19
19–24	12.4	29.9	15, 17	14(2)	18, 20	21, 30	21(2)	20(2)
25–30	12.4	49.9	12(2)	12(2)	10, 11	17(2)	25, 26	23, 25
31	24.7	0	12, 13					
32–37	24.7	9.9	19, 20	17, 19	20, 23	18, 22	18(2)	17, 18
38–43	24.7	19.9	18, 19	19, 23	22, 24	20, 23	19, 21	17, 19
44–49	24.7	29.9	14, 16	15, 18	15, 17	25, 29	22, 23	20, 22
50–55	24.7	39.9	13(2)	14(2)	14, 17	15, 18	20, 23	27, 29
56	49.5	0	11, 12					
57–62	49.5	9.9	18(2)	20, 22	16, 22	21, 23	21, 22	19, 21
63–68	49.5	19.9	18, 19	17, 22	19, 21	23, 27	22, 23	20, 26
69	98.6	0	12(2)					
70–75	98.6	9.9	20(2)	18, 23	21, 25	22, 25	18, 24	16, 18
76	197	0	13(2)					
77–82	197	9.9	22, 23	10, 24	22, 24	23, 24	17, 21	11, 23
83	394	0	14, 17					

*Values in parentheses = No. of animals that died on that day.

Survival times (days) of all mice by treatment: Replication 2

Groups	MTX (mg/kg)	VM-26 (mg/kg)	Survival times by interval between drugs*					
			0 hrs	6 hrs	18 hrs	30 hrs	42 hrs	54 hrs
84	0	0	7(4), 8(4)					
85	0	40						18(2)
86–87	0	50				10, 14	13, 14	
88–93	12.5	40	13, 16	13, 14	10, 20	12, 14	14, 15	16, 18
94–99	25	30	16(2)	17, 18	10, 13	12, 13	14, 15	12(2)
100–105	25	40	9, 10	12(2)	12(2)	10, 14	13, 14	14, 17
106–111	50	20	16, 18	16, 18	18(2)	10(2)	13, 15	14, 18
112–117	50	30	15, 16	15(2)	13(2)	10, 11	11, 14	14(2)
118–123	100	10	15, 26	20, 22	14, 24	12, 13	15, 16	19
124–129	100	20	13, 15	7, 17	15, 16	10(2)	11, 14	11, 13
130–135	100	30	12(2)	15, 17	13, 14	8, 9	11, 12	9, 11
136	200	0	9, 13					
137–142	200	10	8, 9	7, 16	7, 23	13(2)	11(2)	8, 10
143–148	200	20	10, 12	8(2)	8, 10	10, 11	10, 11	9, 11
149–154	200	30	9, 11	11, 16	11, 15	7, 9	9, 12	9, 11
155–160	200	40	8(2)	7, 8	9, 10	9, 11	11(2)	10, 11
161	400	0	7(2)					

*Values in parentheses = No. of animals that died on that day.

Survival times (days) of all mice by treatment: Replication 3

Groups	MTX (mg/kg)	VM-26 (mg/kg)	Survival times by interval between drugs*				
			0 hrs	18 hrs	30 hrs	42 hrs	54 hrs
162	0	0	9(4), 10, 11(2), 16				
163–167	0	35.6	13(2)	14, 27	22, 26	20, 21	19, 21
168–172	0	40.6	11, 12	11, 13	17, 23	21, 26	20, 21
173–177	0	45.7	12, 13	13, 27	16, 17	23, 24	19(2)
178–179	25.4	30.5		17, 26		40+, 25	
180–181	25.4	35.6		14, 15		40+, 34	
182–183	25.4	40.6	11, 12			40+(2)	
184–186	50.8	25.4	15(2)	12(2)		40+, 11	
187–189	50.8	30.5	13, 14	17, 25		12, 24	
190–192	50.8	35.6	12, 13	12, 28		40+(2)	
193–195	102	15.2	25, 35	14, 17		20, 25	
196–198	102	20.3	19(2)	40+, 22		13, 17	
199–201	152	5.08	23, 26	17, 22		17, 25	
202–204	152	10.2	9, 16	25, 30		40+, 18	
205–207	152	15.2	27, 29	25, 26		13, 15	
208	178	0	20, 22				
209–211	178	5.08	18, 19	24, 25		15, 16	
212–214	178	10.2	40+, 27	40+, 16		10, 11	
215	203	0	14, 17				
216–218	203	5.08	40+, 23	11, 12		23, 25	
219–221	203	10.2	12, 13	8, 10		12, 26	
222	229	0	9, 18				
223–224	229	5.08	11, 12			12, 13	

*Values in parentheses = No. of animals that died on that day.

Survival times (days) of all mice by treatment: Replication 4

Groups	MTX (mg/kg)	VM-26 (mg/kg)	Survival times by interval between drugs*					
			0 hrs	20 hrs	25 hrs	30 hrs	35 hrs	40 hrs
225	0	0	8, 9(2), 12					
226–230	31.2	11.7		26, 40+(3)	22, 23, 40+(2)	40+(4)	9, 31, 40+(2)	37, 39, 40+(2)
231–235	46.8	17.5		40+(4)	40+(4)	13, 25, 40+(2)	25, 40+(3)	11, 13, 17, 40+
236–240	70.2	26.3		15, 16, 23, 26	8, 11, 17(2)	9(3), 10	9(4)	9, 10, 11, 12

*Values in parentheses = No. of animals that died on that day.

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Phase I and Pharmacokinetic Study of Menogaril Administered as a 72-Hour Continuous Iv Infusion[1,2]

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Menogaril is a new anthracycline analog of nogalamycin. When administered as a 72-hour continuous iv infusion the dose-limiting toxic effect of menogaril was venous irritation at dose levels that cause only mild leukopenia and minimal gastrointestinal toxicity. Pharmacokinetic studies showed that the rise in plasma concentration during infusion was first-order, with a half-life of 11.9 hours. Total-body clearance of menogaril was 204 ml/minute/m^2. There were no detectable metabolites of menogaril in plasma. Urinary excretion of unchanged menogaril was 17.3% of the dose and *N*-demethylmenogaril was 0.5% over 72 hours. Since menogaril does not appear to be metabolized, a high degree of tissue binding is likely. [Cancer Treat Rep 71:593-598, 1987]

Menogaril is an anthracycline derivative of nogalamycin that has significant antitumor activity in murine P388 and L1210 leukemia and B16 melanoma tumor models (1). In comparison with doxorubicin, menogaril was three times more active in L1210 leukemia and slightly less active in B16 melanoma (1). While doxorubicin interacts strongly with DNA and inhibits DNA and RNA synthesis, menogaril is less avidly bound to DNA and only minimally inhibits DNA and RNA synthesis (2), suggesting a different mechanism of cytotoxicity than doxorubicin. Fluorescence microscopy has shown that menogaril accumulates in the cytoplasm and doxorubicin accumulates in the nucleus, suggesting a different uptake mechanism for menogaril (3). Another difference is increased concentration of menogaril intracellularly relative to doxorubicin at comparable extracellular concentrations. Increased cell kill appears to be related to the increased intracellular concentration of menogaril as demonstrated in B16 melanoma and cultured Chinese hamster ovary cells (3).

Comparative cardiac toxicity studies using the rabbit model have shown that menogaril causes chronic anthracycline cardiomyopathy at cumulative doses > 3000 mg/m^2 comparable to doxorubicin at cumulative doses > 200 mg/m^2, suggesting that menogaril may be 1/15 as cardiotoxic as doxorubicin (4). Since animal tumor models require six to 12 times as much menogaril as doxorubicin to achieve equivalent hematologic toxicity (1), it is possible that therapeutic doses of menogaril will have less cardiotoxicity than doxorubicin in man.

Human plasma compatibility studies (5) suggested that gelation of menogaril occurs at concentrations > 0.15 mg/ml, with potential for venous occlusion if higher concentrations are given rapidly into a peripheral vein. We therefore elected to conduct a phase I trial with menogaril given as a 72-hour continuous iv infusion to ensure maximum dilution of menogaril at the infusion site and to minimize the risk of gelation and phlebitis.

MATERIALS AND METHODS

All patients were > 18 years of age, ambulatory, adequately nourished (> 1500 calories/24 hours), had a life expectancy > 12 weeks, and had histologic or cytologic evidence of advanced, unresectable cancer that was beyond reasonable hope of cure or significant palliation by conventional radiation therapy or chemotherapy. Patients with active cardiac disease (congestive heart failure or arrhythmia requiring drug treatment) were not admitted to the study. Informed consent was obtained for all participants. In addition, all patients were required to have a performance status [Eastern Cooperative Oncology Group (ECOG)] of 0-2 and to have adequate hematologic (wbc count ≥ 4100/mm^3, platelet count ≥ 130,000/mm^3, and Hgb ≥ 10 g/dl), hepatic (normal direct-reacting bilirubin), and renal (serum creatinine ≤ 1.5 mg/dl) functions. Patients were not admitted to the study if they had had major surgery < 30 days prior to entry or if they had had irradiation of >

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1500 cGy to > 30% of bone marrow at any time to avoid compromised bone marrow function. Prior cardiac irradiation did not exclude patient entry. Prior chemotherapy had not been given in the preceding 30 days (42 days for mitomycin and nitrosoureas). Prior treatment with doxorubicin to a cumulative dose < 450 mg/m^2 was permitted but patients who received prior doses between 250 and 450 mg/m^2 were required to have a left ventricular ejection fraction ≥ 50% by multigated analysis scan. Characteristics of the 12 patients entered in this study are shown in table 1. Histologic tumor types are shown in table 2.

TABLE 1.—Characteristics of 12 evaluable patients

Characteristic	No. of patients
Age (yrs)	
Range: 36-68	
Median: 56	
Performance status (ECOG)	
0	2
1	8
2	2
Sex	
Male	6
Female	6
Prior therapy	
None	1
Chemotherapy only	7
Chemotherapy and radiation therapy	4

TABLE 2.—Tumor types

Type of adenocarcinoma	No. of patients
Colorectal	5
Jejunum	1
Pancreas	1
Lung	1
Breast	1
Squamous carcinoma, esophagus	1
Melanoma	1
Sarcoma, synovial	1

Clinical Studies

Prior to initial treatment, all patients were required to have a history and physical examination, cbc, serum chemistry panel [including sodium; potassium; calcium; total protein; phosphate; albumin; glucose; alkaline phosphatase; aspartate aminotransferase (AST); bilirubin, total and direct; creatinine; and uric acid], ECG, chest x-ray, urinalysis, ^{125}I-iothalamate renal clearance, electroencephalogram, and any other studies thought by the investigator to be necessary for evaluation of tumor response. Cbc and chemistry panel were repeated on completion of the menogaril infusion. In addition, cbc was performed twice weekly and serum creatinine and AST once weekly to assess interval toxicity. Cardiac function was evaluated clinically.

Menogaril was obtained as a freeze-dried powder (National Cancer Institute, Bethesda, MD) in 20-mg vials containing 50 mg of menogaril, 100 mg of mannitol, and 16.6 mg of L-lactic acid and was reconstituted with 10 ml of sterile water for injection, USP. The calculated daily dosage of menogaril was further diluted to 1000 ml in 5% dextrose in sterile water and was administered via a peripheral vein at a rate of 41.7 ml/hour using a continuous-infusion pump so as to deliver the entire 1000 ml in a 24-hour period. The planned starting dose was to be 3.5 mg/m^2/24 hours by continuous iv infusion for 3 consecutive days, for a total dose of 10.5 mg/m^2 or approximately 20% of the toxic dose low that was seen in dog toxicity studies on a single-day dose schedule. Prior to activation of the protocol, the starting dose was escalated to 14 mg/m^2/24 hours by continuous iv infusion for 3 consecutive days at the request of the National Cancer Institute drug monitor, since several groups had already treated patients at doses > 50 mg/m^2 by single- or multiple-day rapid infusions. Dosage was escalated successively in groups of three patients according to the following schedule. The previous dose level was doubled unless toxicity was seen. If toxicity was mild but not dose-limiting, subsequent dosage was escalated in 50% increments. When significant (ECOG grade 1-2) but not dose-limiting toxicity was seen, the dosage was escalated in 25% increments. When potentially dose-limiting toxicity was seen in any patient at a given dose level, an additional three patients were treated at that level. If no further toxicity was seen, the dose was escalated by 25% for the next three patients and so forth until moderate (ECOG grade 3) to severe (ECOG grade 4) dose-limiting toxicity was seen in the majority of patients. Assuming hematologic toxicity would be dose-limiting as with other anthracycline drugs, our intent was to escalate the dosage successively until wbc and/or platelet count nadirs were reached (< 3000/mm^3 and/or < 100,000/mm^3, respectively) without severe myelosuppression (wbc count < 1000/mm^3 and/or platelet count < 50,000/mm^3).

Patients were re-evaluated at 5-week intervals and retreated at the same dose level if they had no significant toxicity during the preceding course of treatment and they showed clinical improvement with no objective evidence of progression. There was no provision to escalate the dosage at re-evaluation in the absence of significant toxicity.

Pharmacokinetic Studies

Prior to initiation of menogaril infusion, all patients had 5 ml of heparinized venous blood collected from an indwelling cannula in the arm opposite to that used for the menogaril infusion. After starting the infusion, 5-ml samples of heparinized venous blood were collected at ¼, ½, ¾, 1, 1½, 2, 4, 6, 8, 12, 18, 24, 32, 40, 48, 56, 64, and 72 hours and immediately chilled. Plasma was separated by centrifugation and was stored frozen at −70°C. Urine was collected before starting menogaril infusion and in 24-hour aliquots for the 3 days of infu-

sion. The 24-hour volume was recorded and 20-ml aliquots were stored frozen at −70°C.

Plasma or urine (1 ml) was mixed with 0.1 ml of 0.1 M glucuronic acid, 0.5 g of *N,N*-dimethyldaunorubicin internal standard, and 3 ml of acetonitrile:methanol, 1:1 (vol/vol), and centrifuged at 2500 rpm for 30 minutes at −5°C. The supernatant was removed, evaporated under N_2 at room temperature to about 0.5 ml, and 0.25 ml was taken for high-performance liquid chromatography (HPLC). Menogaril concentrations were determined by a modification of the HPLC method of McGovren et al (6) using an RP-2 HPLC column with 30% acetonitrile in 0.3 M acetic acid and 15 mM heptane sulfonic acid mobile phase at a flow rate of 1.5 ml/minute with fluorescence detection (excitation, 254 nm; emission, > 550 nm). The limit of detection of menogaril was 5 ng/ml of plasma. Serial plasma menogaril concentrations were plotted over time and values for plasma half-life, steady-state plasma concentration, volume of distribution, and total-body plasma clearance were calculated using the NONLIN least-squares regression analysis computer program (7). Urine concentrations of menogaril and its metabolite, *N*-demethylmenogaril, were determined at 24 and 72 hours into the infusion.

RESULTS

Phase I

Three patients received menogaril (table 3) at a starting dose level of 14 mg/m²/day × 3 (total dose, 42 mg/m²) by continuous iv infusion. None experienced gastrointestinal toxicity. One patient developed burning pain at the infusion site followed by skin and sc edema surrounding the infusion cannula. No sign of extravasation was noted and the infusion cannula was changed. There were no long-term sequelae such as ulceration or sclerosis of the involved skin. This complication occurred daily and on each occasion rapidly resolved after changing the cannula site. No hematologic toxicity was seen.

The dose of menogaril was escalated to 28 mg/m²/day × 3 (total dose, 84 mg/m²) by continuous iv infusion in three patients. One of the three patients experienced mild, transient leukopenia (wbc count, 3000/mm³ on Day 17) (table 3). All three patients previously received treatment with mitomycin-containing regimens. No thrombocytopenia was seen and the leukopenia rapidly resolved. No gastrointestinal toxicity was seen. Two of the three patients experienced pain and edema at the infusion site which required changing the infusion cannula site two to three times daily. One of the patients developed transient erythema along the vein but this resolved rapidly without a palpable cord. The infusion volume was increased to 2000 ml/24 hours in the third patient and no venous irritation was noted.

Six patients were treated with menogaril at a dose level of 42 mg/m²/day × 3 (total dose, 126 mg/m²) in 2000 ml/24 hours. Four of the six patients experienced leukopenia of < 3000 cells/mm³ (range, 1800-2700) (table 3). The patient with nadir leukopenia of 1800 cells/mm³ had previously received whole-pelvis irradiation to 4500 cGy. Two other patients had had prior mitomycin-containing regimens. Two patients had no significant leukopenia although one developed leukopenia (wbc count, 3500/mm³) after a second course of menogaril. In seven treatment courses the mean wbc count nadir was 3300/mm³. The mean time of nadir was Day 13 (range, 9-16 days). Complete recovery was seen in 5-7 days. No thrombocytopenia was seen. Mild nausea and emesis was experienced by one patient.

TABLE 3.—Toxicity (all courses)

Patient No.	Prior radiation therapy	Prior chemotherapy*	Dose (mg/m²/72 hrs)	Wbc count nadir		Platelet count nadir	
				Cells/mm³	Day	Cells/mm³	Day
1		TG, 5-FU, CP	42	4700	17	418,000	14
2		5-FU	42	7300	18	214,000	20
3		5-FU	42	4400	6	298,000	6
4		CFP, VAM	84	3000	17	236,000	15
5	Abdomen and pelvis (2000 cGy)	TCNP, MOF-Strep	84	5100	12	373,000	12
6		IFL, Mito C	84	12,600	9	500,000	9
7	Pelvis (4500 cGy)	5-FU + PALA	126	1800	15	372,000	12
8		TG	126	5000	14	280,000	7
			126†	3500	16	275,000	7
9			126	2000	9	139,000	9
10	Rib (3000 cGy)	CAP, MCM, VP16	126	2200	13	276,000	13
11	Shoulder (5000 cGy)	MAP	126	2700	13	330,000	9
12		TG, CCNU, 5-FU, CP	126	5750	11	422,000	11

*TG = 6-thioguanine; 5-FU = 5-fluorouracil; CP = cisplatin; CFP = cyclophosphamide, 5-fluorouracil, and prednisone; VAM = vinblastine, doxorubicin, and mitomycin; TCNP = tricyclic nucleoside 5'-phosphate; MOF-Strep = streptozocin, semustine, vincristine, and 5-fluorouracil; IFL = recombinant leukocyte interferon alpha-2A; Mito C = mitomycin; CAP = cyclophosphamide, doxorubicin, and cisplatin; MCM = mitomycin, lomustine, and methotrexate; VP16 = etoposide; MAP = mitomycin, doxorubicin, and cisplatin; and CCNU = lomustine.

†2nd course.

Venous irritation with erythema, burning pain, and tissue edema at the infusion site was nearly universal at the 42-mg/m^2/day × 3 dose level despite infusion volume of 2000 ml/24 hours and necessitated changing infusion sites every 3–4 hours for the duration of the infusion. Patient 11 terminated the infusion 4 hours prior to completion due to pain at the infusion site and anxiety relating to the frequent cannula changes. Although Patients 5, 8, and 11 developed erythema along the course of the vein, sclerosis and palpable cord were not seen. Cutaneous ulceration was not experienced by any patient.

Abnormalities of hepatic or renal function were not seen in any patient at any dose level. Cardiac toxicity was not seen, although only one patient received a second dose of menogaril and that patient progressed after the second dose. No patient received sufficient menogaril to warrant cardiac function testing and no patient experienced palpitations, dyspnea, or signs of congestive heart failure during the infusion.

Pharmacokinetics

Menogaril pharmacokinetic studies were determined in nine patients: two at 42 mg/m^2/72 hours, one at 84 mg/m^2/72 hours, and six at 126 mg/m^2/72 hours. Steady-state plasma concentrations were achieved within 24 hours of starting the continuous iv infusion (fig 1). Metabolites of menogaril could not be identified in the plasma over the 72-hour sampling period (fig 2). Plasma concentrations of menogaril (table 4) increased, with a mean half-life of 11.9 hours (range, 4.3–18.3). Mean volume of distribution was 197 L/m^2 (range, 89–359). Mean total-body plasma clearance was 204 ml/minute/m^2 (range, 120–325). Dose-dependent elimination of menogaril was not seen. Urinary excretion of menogaril was 10.7% of the administered dose in 24 hours and 17.3% in 72 hours. Negligible amounts (< 0.6% of the total dose in 72 hrs) of the principal metabolite *N*-demethylmenogaril were seen in the urine.

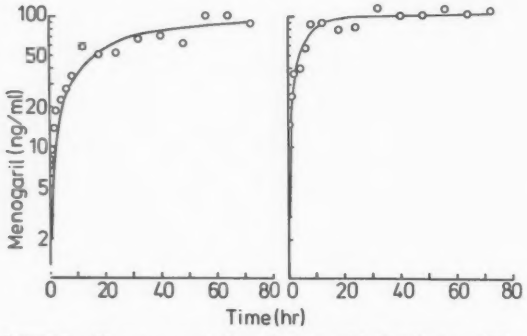

FIGURE 1.—Plasma concentrations of menogaril in 2 patients receiving a continuous 72-hr infusion of menogaril at 42 mg/m^2/day. Continuous lines are computer-generated fits to the data assuming a 1-compartment open model.

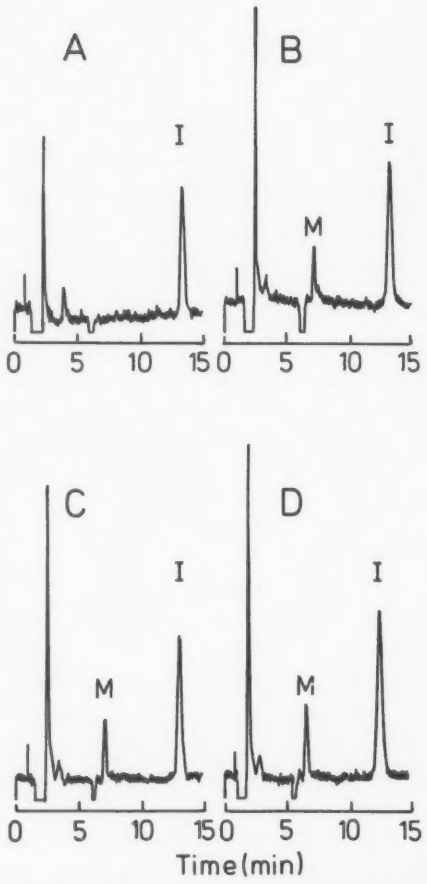

FIGURE 2.—Chromatograms of plasma from a patient receiving menogaril at 42 mg/m^2/day, by continuous 3-day infusion. HPLC was an RP-2, 5-column with 30% acetonitrile with 0.3 M acetic acid and fluorescence detection (254 nm excitation; > 550 nm emission). I = *N,N*-dimethyldaunorubicin internal standard; M = menogaril; A = 0 hr; B = 12 hrs, 89 ng/ml; C = 48 hrs, 100 ng/ml; and D = 72 hrs, 111 ng/ml.

DISCUSSION

Menogaril is an interesting analog of nogalamycin for investigation as an antineoplastic drug because of its similarities to doxorubicin in the murine tumor drug screens (1), its apparent differing mechanism of action (2,3), and reduced cardiac toxicity in the rabbit model (4).

The relative lack of gastrointestinal toxicity of menogaril and selective hematologic toxicity (leukopenia without thrombocytopenia) suggests that this drug is worthy of phase II investigation and may be of particular interest in hematologic malignancies.

Venous irritation was dose-limiting in our study. Mild to moderate leukopenia was seen at the highest dose level studied (126 mg/m^2/72 hrs). Further dose escala-

TABLE 4.—Pharmacokinetics

Patient No.	Dose (mg/m^2/day)	Plasma kinetics*				Urinary excretion (%)			
						Menogaril		Metabolite†	
		C_{ss} (ng/ml)	$t_{1/2}$ (hrs)	Vd (L/m^2)	Cl ($ml/min/m^2$)	24 hrs	72 hrs	24 hrs	72 hrs
1	14	53	18.3	209	182.3	8.4	22.5	0.33	0.84
3	14	81	8.5	89	120.3	14.3	10.10	0.35	0.21
5	28	150	15.4	170	127.1	10.6	—	0.0	—
7	42	141	10.1	182	208.1	0.2	13.4	0.24	0.50
8	42	102	4.3	109	286.6	5.9	18.0	2.29	0.89
9	42	118	12.3	265	247.3	22.8	23.1	2.28	1.06
10	42	194	9.8	127	150.0	16.1	22.8	0.29	0.15
11	42	144	15.8	264	193.1	10.5	11.1	0.09	0.13
12	42	90	12.8	359	324.9	7.4	18.0	0.17	0.35
Mean ± SEM			11.9	197	204.4	10.7	17.3	0.67	0.52
			1.4	29	23.5	2.2	1.9	0.31	0.13

*C_{ss} = steady-state plasma concentration; $t_{1/2}$ = plasma half-life; Vd = volume of distribution; and Cl = total-body plasma clearance.
†No *N*-demethylmenogaril was detectable in plasma.

tion may have been tolerated from the standpoint of hematologic toxicity but in our opinion would have required administration via central venous catheter due to burning and edema at the infusion site requiring excessive rotation of infusion sites. Although venous irritation has been described in other phase I trials when menogaril was given by short infusion, it was not dose-limiting (8). The etiology of venous irritation when menogaril is administered by continuous iv infusion is uncertain but does not appear to be secondary to precipitation or venous occlusion since all infusion lines remained patent and no phlebitic cords developed. Doubling the infusion volume ameliorated this toxicity at 84 mg/m^2/72 hours but was not of benefit at 126 mg/m^2/72 hours.

The rise in menogaril plasma concentration during the infusion was best described by a first-order process, with a mean half-life of 11.9 hours. This is similar to the plasma elimination half-life of menogaril seen in the mouse [10.6 hours (9)] and longer than the elimination half-life seen in the dog [3.3 hours (10)] and the rabbit [2.7 hours (11)]. We did not detect an initial distributive phase for menogaril, as has been reported in animals (9,10), possibly because sufficient data points were not collected during the early stages of the infusion. Our first blood sample was obtained 15 minutes into the infusion and would have missed an α-distribution phase of 10–15 minutes. The mean total-body clearance of menogaril in man was 204 ml/minute/m^2 compared to 91.2 ml/minute/m^2 in mouse (9) and 47 ml/minute/m^2 in rabbit (11). We were not able to detect menogaril metabolites in plasma. This is similar to findings in rabbits (11) but differs from mice (9) and dogs (10), where *N*-demethylmenogaril can account for up to 15% of simultaneously measured menogaril concentrations. Urinary excretion of unchanged menogaril was low in man, being 10.7% of the dose in 24 hours and 17.3% in 72 hours; only low levels of *N*-demethylmenogaril were found in urine. The lack of apparent metabolism of menogaril by man is interesting. It has been reported that *N*-demethylmenogaril, at its optimum dose, is tenfold more potent than menogaril in prolonging the lifespan of mice bearing P388 leukemia and B16 melanoma and may contribute to the observed antitumor activity of menogaril in mice (12).

Studies with Chinese hamster ovary cells in culture indicate that continuous exposure of exponentially growing cells for 24 hours causes effects on cell cycle and degrees of cell kill similar to those produced by exposures of 2 and 7 hours. In the presence of menogaril, cells progressed normally from M to G_1 and G_1 to S during the cell cycle. The S phase is prolonged and there is a block produced at the G_2/M interface at concentrations ranging from 0.2 to 1.0 μg/ml (13). These data suggest no advantage to continuous-infusion therapy at the level of cellular kinetics.

At all dose levels studied in this phase I trial, the volume of distribution was large (89–359 L/m^2). Total-body plasma clearance averaged 204.4 ml/minute/m^2. No evidence of metabolism of significant amounts of menogaril was found on HPLC analysis of plasma and urine. These data are compatible with rapid uptake of menogaril by cells, with extensive protein binding and with gelation of the drug within the vascular system. Our present data do not allow us to distinguish among these possibilities.

We selected the continuous-infusion regimen to deliver dilute solutions of menogaril to minimize the possibility of intravascular gelation and to minimize venous irritation given the preclinical observation of low solubility and gelation in ionic media and our previous experience with bisantrene (14,15). Although frank thrombophlebitis was not encountered in this study, severe annoying venous irritation with pain and erythema over and around the course of infused vessels was dose-limiting and necessitated unacceptably frequent changes of infusion site. Other than peripheral venous irritation, menogaril given by 72-hour infusion was associated with little toxicity. Mild to moderate hematologic toxicity was

encountered at the maximal dose evaluated, 126 mg/m²/72 hours. Three of the four patients who experienced leukopenia previously had had irradiation to at least 10% of their bone marrow and were previously treated with mitomycin or lomustine. We elected not to study the drug at higher doses by continuous infusion because to do so would have required administration by central venous line. We saw no therapeutic advantage of continuous-infusion over short-infusion results obtained by others (16) with regard to plasma pharmacokinetics or hematologic toxicity and thus could not justify the added expense and morbidity of central venous catheter placement in this phase I study. If significant antitumor activity is seen in phase II trials and cardiac toxicity is seen, then further studies of menogaril may be considered via central venous catheter to further define the maximum tolerated dose and role of continuous infusion in reducing cardiac toxicity. The fact that no antitumor activity was seen in this study must be considered in light of the possibility that conservatively larger doses of the drug may be tolerable given by infusion into larger vessels.

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Pharmacokinetics of High-Dose Teniposide[1]

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This paper describes the pharmacokinetics of teniposide (VM-26) after being administered iv in high doses to eight cancer patients (maximum dose, 1.0 g/m^2). VM-26 levels in plasma, urine, saliva, duodenal fluid, and cerebrospinal fluid were determined using high-performance liquid chromatography in combination with electrochemical detection. The plasma concentration-time curve of VM-26 showed a triphasic decay with a slow third phase in five patients, whereas in two patients the plasma concentration decay was biphasic. The plasma pharmacokinetics of VM-26 proved to be linear and could be fitted to a three-compartment model (five patients) and to a two-compartment model (two). The steady-state volume of distribution varied from 13.2 to 24.7 L/m^2. The total-body clearance ranged from 5.84 to 10.18 ml/minute/m^2. Low concentrations of VM-26 were found in saliva, duodenal fluid, cerebrospinal fluid, and urine. Excretion of unchanged VM-26 into the urine varied from 8.8% to 13.9% of the administered dose. No glucuronide of VM-26 could be detected in plasma or other biological fluid. [Cancer Treat Rep 71:599-604, 1987]

Teniposide (VM-26), a semisynthetic derivative of podophyllotoxin, is being used more and more in the treatment of cancer patients (1). Despite a number of reports (2-5), the pharmacokinetics of VM-26 in man have not been fully elucidated. So far, two- and three-compartment models (2,3,5) and a noncompartment model (4) have been described.

Phase I studies showed that the therapeutic efficacy of VM-26 and etoposide are schedule- and dose-dependent (6). The schedule-dependent effect may possibly be reflected in the disposition of VM-26, which may in fact be also schedule-dependent. To reveal such a phenomenon we studied the pharmacokinetics of high-dose VM-26 (7). To define the pharmacokinetic parameters, we determined VM-26 levels in plasma, urine, saliva, duodenal fluid, and cerebrospinal fluid (CSF) over a 10-day period.

MATERIALS AND METHODS

Patients.—Eight patients (34-63 years of age; five males and three females) with persistent or progressive malignant disease (eg, sarcoma, breast cancer, ovarian cancer, small cell lung cancer, adenocarcinoma, and squamous cell lung cancer) were entered in the study. All patients had a Karnofsky score > 50 and gave informed consent for participation in a phase I study including pharmacokinetic studies. All patients had normal renal and liver functions as were indicated by normal bilirubin, serum creatine, and normal values of SGOT and alkaline phosphatase.

Drug treatment.—A total dose of VM-26 of 0.3, 0.6, or 1.0 g/m^2 was administered over 3 days. One-third of the total dose was diluted in 1 L of normal saline (maximum drug concentration, 0.8 mg/ml) and was administered iv over a period of 2 hours at 0, 24, and 48 hours. The regimen was administered to seven patients; one additional patient (No. 8) received a single dose of 170 mg of VM-26.

Sample collection.—Blood samples of 5 ml were collected in heparinized tubes. For preparation of plasma the blood was promptly centrifuged for 10 minutes at 4°C. The samples were taken at 0, 1, 1.5, 2, 3, 4, 6, and 12 hours after each drug administration and at 24, 36, 48, 60, 84, and 108 hours after the last administration. In two patients (Nos. 1 and 2) the last blood sample was taken 48 hours after the last infusion. Exact sampling times were used for the pharmacokinetic modeling. Urine was collected in 6-hour periods for a total of 144 hours after the start of the first infusion. Saliva

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samples were collected from two patients at the same time as the plasma samples. The patients were asked to chew on a Teflon tablet and 2 ml of the produced mixed saliva was stored in a tube. Duodenal fluid was collected in an additional patient (No. 8) from the proximal part of the duodenum through a polyvinyl chloride tube, which had already been inserted for nutritional purpose. The duodenal fluid samples were collected at 1, 2, 3, and 4 hours after the start of the infusion. In one patient (No. 3), CSF samples of 1.5 ml were taken from the Ommaya reservoir at 2.0, 2.5, 2.9, 3.9, 5.5, 22.6, 25, 25.3, and 29.3 hours after the start of the first administration. All samples were frozen and stored at −20°C until taken for analysis.

Drug assay.—VM-26 was quantified by high-performance liquid chromatography with electrochemical detection. The method used was a variant of the assay used for etoposide (8). For the analysis of VM-26 in concentrations < 2 μg/ml, 1.0 ml of plasma or other biological fluid was extracted after the addition of the internal standard (etoposide) with 2.0 ml of 1,2-dichloroethane (DCE). For the analysis of higher concentrations, 0.1 ml of the biological sample was extracted with 1.0 ml of DCE after addition of the internal standard. After extraction, the organic phase was evaporated and the residue was dissolved in 200 μl of the mobile phase by sonication for 6 minutes. Amounts of 5–150 μl of the solution were injected into the chromatographic system. Standard samples, in the corresponding biological fluids, were prepared by spiking drug-free biological samples. Repetitive extraction of VM-26 containing bile and CSF samples of patients revealed that the extraction recovery was almost complete after the first extraction with DCE. Because of this and the lack of blank CSF and bile specimens, a 0.1 M, pH 7.0, phosphate buffer was used as matrix for calibration. The calibration curves were processed for each set of patient samples. The chromatographic system in combination with the electrochemical detector has been described previously (8). For the chromatography of VM-26 a Phenyl μBondapak (10 μm, 30 cm × 4.6 mm internal diameter) column was used. The mobile phase consisted of methanol 0.065 M phosphate buffer, pH 7.0 (50/50 or 55/45 wt/wt). A flow of 1.0 ml/minute was used. With this method concentrations as low as 5 ng of VM-26/ml of plasma could be determined. Repeated analysis of VM-26-spiked plasma samples ($n = 5$) (concentrations of 50 ng and 12.5 μg/ml) showed recoveries of 95.8% and 97.3%, respectively, with relative standard deviations of 3.2% and 2.5%, respectively. Repeated analysis of patient samples [urine (8.43 μg/ml) and plasma (21.19 μg/ml)] showed relative standard deviations of 3.9% ($n = 6$) and 2.3% ($n = 5$), respectively. The three calibration curves, covering a concentration range of 0.005–150.0 μg of VM-26/ml of plasma and 2.0–150.0 μg of VM-26/ml of urine, were linear. The intercepts of these calibration curves were not significantly different from zero ($P = 0.05$). All correlation coefficients were at least 0.994.

Pharmacokinetic data analysis.—The pharmacokinetic data analysis was performed with a NONLIN computer program (9) extended with a Dirac Delta function so that it fitted the three VM-26 plasma concentration-time curves in one run (10). The reciprocal of the plasma concentration was used as a weighting factor. From the curve fitting the following parameters were obtained: V_c = the volume of the central compartment, and k_e = the apparent first-order elimination rate constant from the central compartment and the apparent first-order intercompartmental transfer rate constants k_{12}, k_{21}, k_{13}, and k_{31}. The apparent first-order rate constants α, β, and γ were calculated as described by Gibaldi and Perrier (11). The area under the concentration versus time curves (AUC) and the area under the moment curve [AUMC = (c × t) versus t] were calculated up to the last measured plasma sample using the linear trapezoidal method. The noncompartmental total-body clearance (Cl_{tot}), the steady-state volume of distribution (Vd_{ss}), and the mean residence time (MRT) were calculated as described by Perrier and Mayersohn (12) for multiple-dose administrations. The renal clearance (Cl_{ren}) of unchanged VM-26 was calculated by dividing the total excreted amount (milligrams) by the plasma AUC (table 1).

RESULTS

Table 1 shows the pharmacokinetic parameters calculated with the fitting procedure. All patients received three infusions of the drug over 3 consecutive days. In seven patients the plasma concentration-times after the first and second infusions led to pharmacokinetics which could be described by a two-compartmental model.

However, in five patients after the third infusion, the log plasma concentration versus time curve showed a triphasic decay which fitted best to a three-compartment model. After the third infusion of the two other patients, the log plasma concentration curves were biphasic and best described by a two-compartmental model. The plasma level of VM-26 at 7 days after the first infusion varies from 20 to 250 ng/ml (median, 130). The linear relation between the total plasma AUC and the total administered dose is shown in figure 1. The recovery of unchanged VM-26 in the urine varied from 8.8% to 13.9% (mean, 11.8%; SD, 2.0%). In all patients a plateau in the urinary excretion of unchanged VM-26 was reached within 24 hours of the third infusion.

Careful examination of the urine and plasma extracts by means of the described chromatographic system with electrochemical and ultraviolet detection (280 nm) revealed no trace of possible metabolites, although urine and plasma did contain very low amounts of *cis*-VM-26. When the urine was incubated with β-glucuronidase in 0.2 mM acetate buffer (pH 5.0) (2000 units of β-glucuronidase Bl/ml, Sigma Chemical Co.), the VM-26 concentration measured did not increase.

The ratios of the saliva and plasma concentrations were measured in two patients. The relation between the plasma and saliva levels is shown in figure 2. The measured saliva level proved to be 0.37% ± 0.23% SD

TABLE 1.—Pharmacokinetic parameters*

Patient No.	$t_{½\alpha}$ (hrs)	$t_{½\beta}$ (hrs)	γ (hrs^{-1})	Vd_{ss}† (L/m^2)	Cl_{tot}‡ ($ml/min/m^2$)	Cl_{ren}§	MRT¶ (hrs)	Total dose (g/m^2)
1	2.91	17.20	—	15.1	6.01	n.d.	41.7	0.3
2	1.01	20.27	—	13.2	7.14	0.62	30.8	0.6
3	1.72	6.41	6.129×10^{-5}	13.7	8.43	0.87	27.0	0.6
4	3.34	11.99	2.416×10^{-5}	14.1	6.47	0.78	35.4	0.6
5	2.75	14.23	1.354×10^{-5}	24.7	10.18	1.35	40.4	1.0
6	2.47	24.16	0.553×10^{-5}	14.5	5.84	0.74	41.5	1.0
7	3.14	15.40	1.799×10^{-5}	21.0	8.61	1.20	40.7	1.0
Median	2.75	15.40	1.799×10^{-5}	14.5	7.14	0.83	40.4	—
Mean	2.48	15.67	2.45×10^{-5}	16.6	7.53	0.93	36.8	—
SD	0.83	5.73	2.17×10^{-5}	4.4	1.60	0.29	5.9	—

*T = infusion time (hrs); k_0 = 0-order infusion rate constant (mg/hr); D = dose in mg = k_0.T; B.A. = body area in m^2; and X_u = amount (mg) of unchanged VM-26 excreted into the urine.

$$\dagger Vd_{ss} = \frac{D}{AUC} \times MRT.$$

$$\ddagger Cl_{tot} = \frac{\text{Total D}}{\text{Total AUC} \times \text{B.A.}}.$$

$$\S Cl_{ren} = \frac{X_u}{\text{Total AUC} \times \text{B.A.}}.$$

$$\P MRT = \frac{AUMC}{AUC} - \frac{\Sigma_0 \int^\infty X.dt}{\Sigma D}$$

$$\frac{\Sigma_0 \int^\infty X.dt}{\Sigma D} = \frac{(½.k_0.T^2)_1 + (½.k_0.T^2)_2 + (½.k_0.T^2)_3}{(k_0.T)_1 + (k_0.T)_2 + (k_0.T)_3}$$

($n = 22$) of the simultaneously determined plasma level in Patient 4 and 0.42% ± 0.20% SD ($n = 29$) in Patient 6. Of VM-26 bound to plasma albumin (1-saliva/plasma), 100% can be calculated. The measurement revealed that in both patients 99.6% of the concentration is bound to plasma albumin.

Figure 3 presents the log plasma concentration and log CSF concentration versus time curves after the administration of VM-26 infusions at 0, 24, and 48 hours in Patient 3.

The measured CSF levels (10–59 ng/ml) proved to be 0.03%–0.55% of the simultaneously determined plasma level (mean, 0.17%; SD, 0.16%; $n = 8$). The excretion of VM-26 by the bile into the duodenal lumen was studied by measuring the VM-26 level in plasma and duodenal fluid after administration of a single dose of 170 mg of

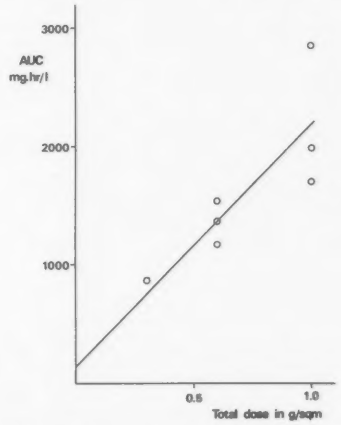

FIGURE 1.—Relation between total administered dose (g/m^2) and total AUC (mg · hr/L) ($r^2 = 0.909$).

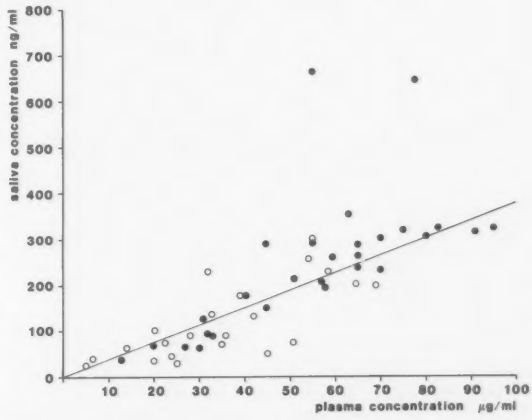

FIGURE 2.—Relation between VM-26 concentrations in plasma and saliva samples of Patient 4 (○, $n = 22$) and Patient 6 (●, $n = 29$).

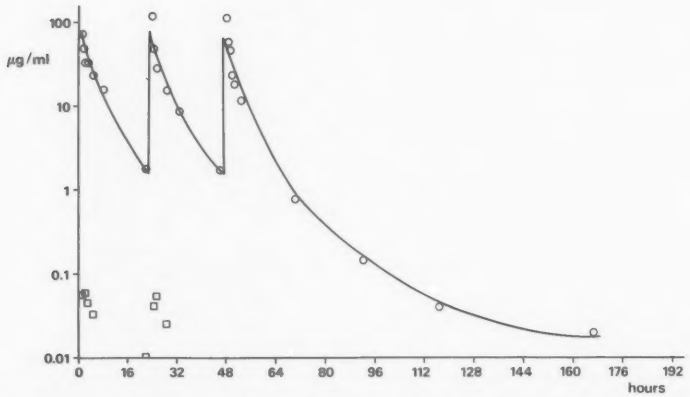

FIGURE 3.—Log plasma (○) and log CSF (□) concentrations vs time curve after administration of 3 consecutive doses of VM-26 (350 mg/day) to Patient 3.

VM-26 to Patient 8. Figure 4 shows the course of VM-26 concentrations in plasma and duodenal fluid in this patient.

DISCUSSION

The pharmacokinetics of VM-26 were studied because of the equivocal results and high interindividual variability of the plasma pharmacokinetics reported in studies of different schedules and dose levels of VM-26 (2,4,5). The analytical method used in this study permits the measurement of VM-26 levels as low as 5 ng/ml of plasma. Therefore, it is possible to detect the low plasma levels present in the third phase of the pharmacokinetic curve. This third phase is possibly due to the elimination of VM-26 from a deep peripheral compartment. This hypothesis is in good agreement with the fact that only 44.5% of the total administered dose was recovered in the urine as unchanged drug and metabolites within 72 hours (2). This indicates that part of the administered amount of VM-26 is sequestered in various tissues and is redistributed very slowly into the plasma compartment.

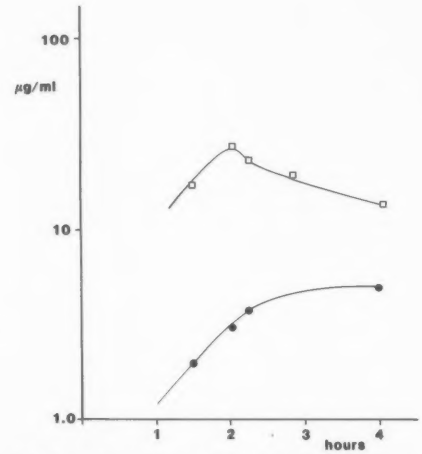

FIGURE 4.—Log plasma (□) and log-duodenal fluid (●) concentrations time curve after administration of a single dose of 170 mg (0.3 g/m^2) of VM-26 to Patient 8.

This slow terminal phase was not detected in two patients because the sampling time was too short. In these patients the last plasma samples were taken at t = 96 hours.

Because of the relatively short time period during which the third phase was observed in the other five patients, it is not realistic, with the limited observations available, to calculate the half-life of this phase or to extrapolate to infinity to determine the AUC and AUMC.

The linear relationship between the total AUC and the total dose (fig 1) and the absence of cumulation in the plasma levels indicated linear pharmacokinetics on the disposition and elimination of VM-26 at a dose level $\leq$ 1.0 g/m^2. Linear pharmacokinetics were also found for high-dose etoposide (13). The total-body clearance in this study is lower than the total-body clearance found after the administration of VM-26 in low doses (30 mg/m^2) (3) or in high doses as a continuous infusion (4). The observed renal clearance is in the same range as previously reported (2,4). This indicates that the difference in the total-body clearance is due mainly to a difference in the metabolic clearance or excretion of VM-26 and/or metabolites by the bile. The low value of total-body clearance results in a high MRT.

Our study demonstrates poor diffusion (fig 3) of VM-26 into the CSF. Assuming that the plasma protein binding is as high as 99.6% (2), it can be expected that after the plasma distribution phase the concentrations of VM-26 in the CSF will approach 0.4% of the plasma concentration; however, the mean concentration in CSF was 0.16%. From this it can be concluded that not only does

the protein binding of VM-26 play a significant role in the diffusion of VM-26 but the permeability of the blood-brain barrier for VM-26 is important as well. Measurements of VM-26 in tissue of a brain tumor, however, indicated that VM-26 reaches active concentrations in brain tumors (14). This might indicate that CSF levels of VM-26 are not representative for the VM-26 levels in brain tumors (14). Under physiological circumstances VM-26 is a neutral compound. The extent of its distribution into saliva depends only on the binding to plasma protein. As expected, VM-26 levels in the saliva were much lower than the VM-26 levels in plasma.

The saliva/plasma concentration ratio calculated in the two patients shows a high variation (fig 2). If it is assumed that the saliva concentration represents the free fraction in the plasma, the percentage of VM-26 bound to plasma albumin can be calculated. The values observed in both patients are in good agreement with the binding of 99.6% found in vitro (2). It is probable that the high protein binding of VM-26 causes the low concentrations in the saliva. This in turn may be responsible for the absence of mucositis of the oropharynx. The latter is a pronounced side effect when etoposide is administered in high doses.

VM-26 is excreted slowly by the bile into the duodenum. Despite the high plasma protein binding of VM-26 the levels reached in the bile are high. From this it can be concluded that the VM-26 is excreted actively in the bile. The VM-26 excretion by the bile into the duodenum could be the start of an enterohepatic circulation. However, the plasma concentration versus time curve showed no evidence of the adsorption of VM-26 from the intestine. It can be concluded that high doses of VM-26 (≤ 1.0 g/m^2) can be administered without disturbance of linear pharmacokinetics and without cumulation of the amount of VM-26 in plasma.

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Combination Chemotherapy in the Treatment of Adenocarcinoma of Unknown Primary Origin[1]

Natalio Walach* and Yoav Horn[2]

A total of 21 patients with histologic diagnosis of metastatic adenocarcinoma and an occult primary tumor were treated with combination chemotherapy. The regimen consisted of cyclophosphamide, vincristine, methotrexate, and 5-fluorouracil (COMF). The overall response rate was 47.6%. Six patients (28.6%) achieved complete response (mean duration, 36.5 months) and four patients (19%) achieved partial response (mean duration, 7.7 months). Toxicity was manageable and occurred in 14 patients (67%). The COMF combination serves as a broad-spectrum chemotherapy regimen in adenocarcinoma of unknown primary origin. [Cancer Treat Rep 71:605-607, 1987]

Metastatic adenocarcinoma of unknown primary origin (ACUP) represents a clinical entity with diagnostic and therapeutic problems. Metastatic spread may involve one or more sites, such as liver, lungs, bones, brain, lymph nodes, pleura, or peritoneal cavity (1). The potential diversity of tumor cells originating from different organs results in the necessity to establish a wide-range treatment regimen to include all possible primary tumor cells.

The anatomic site of origin cannot be determined in approximately 0.5%-10% of all diagnosed cancer patients (2). In our department this incidence reaches 0.7% of all referred cancer patients. Preliminary experience with ACUP showed potential therapeutic benefit of the cyclophosphamide, vincristine, methotrexate, and 5-fluorouracil (COMF) regimen (3).

The present study was undertaken to investigate the efficacy of a combination of four cytotoxic drugs, COMF, in the treatment of ACUP.

MATERIALS AND METHODS

The diagnosis of ACUP was established after histologic confirmation from a metastatic site revealed adenocarcinoma, and all relevant performed tests could not establish the site of the primary tumor. These tests included complete physical examination, hematologic workup including wbc count, platelet count, and hemoglobin, and biochemical levels including urea, electrolytes, creatinine, bilirubin, serum proteins, cholesterol, alkaline phosphatase, CEA, and gamma glutamyl transpeptidase.

Other pretreatment studies included chest x-ray, barium meal and barium enema, abdominal ultrasound, scan of the liver, bones, and thyroid, and computerized tomographic (CT) scan of the chest, abdomen, and brain (4). If clinically indicated, serum acid phosphatase was measured.

Mammograms were not routinely performed. However, to rule out a primary breast tumor, the breasts were examined prior to therapy and at frequent intervals thereafter by two experienced senior physicians, including transillumination of the organ. This method proved, in our hands, to be highly accurate in evaluation of breast cancer in general and in this group in particular. Mammograms were carried out only if either test revealed some level of uncertainty.

The criteria for patient's entry into this study included an obscure primary tumor site (despite all tests done as mentioned above), an estimated life expectancy of at least 3 months, no previous radiation therapy or chemotherapy, presence of measurable or evaluable lesions, and performance status of at least 50% on the Karnofsky performance scale (KPS).

THERAPY

The chemotherapy regimen (COMF) was administered iv as follows: cyclophosphamide, 250 mg/m^2, on Days 1 and 5; vincristine, 1.0 mg/m^2, on Days 2 and 5; methotrexate, 25 mg/m^2, on Days 1 and 4; and 5-fluorouracil, 500 mg/m^2, on Days 1-5.

Treatment courses started within 3 weeks of diagnosis and were repeated at 28-day intervals. In patients > 65

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TABLE 1.—Dose modifications for hematologic toxicity

Level	Wbc count (cells/mm^3)	Platelet count (cells/mm^3)	Dose (%)
0	> 4000	> 100,000	100
1	3000-3999	75,000-99,999	50
2	2000-2999	50,000-74,999	25
3	< 2000	< 50,000	0

years of age, treatment doses were reduced by one-third due to our previous experience with this patient population in which increased intolerance was frequently observed.

Dose modifications for hematologic toxicity are summarized in table 1. Oncovin dosage was reduced or completely discontinued if neurological toxicity such as paresthesia and loss of deep tendon reflexes became apparent. In cases with stomatitis, the methotrexate dosage was monitored accordingly. In cases with increased frequency of diarrhea, 5-fluorouracil was temporarily discontinued.

Repeated follow-up examinations were carried out prior to each therapy course. These included complete physical examination, measurement of apparent lesions, and hematologic and biochemical blood tests.

The criteria for response were as follows: *complete response* (CR)—complete disappearance of all objective evidence of tumor, with no development of new lesions for ≥ 4 weeks; *partial response* (PR)—a 50% reduction of measurable lesions in at least two dimensions for at least 4 weeks; *no change*—measurable lesions unchanged for at least 4 weeks without any new signs or symptoms of disease; and *disease progression*—increase in diameter of measurable lesions of at least 25%, or appearance of new lesions.

RESULTS

The site of diagnosis, sex, age, and KPS score for the 21 evaluable patients are summarized in table 2. There were 14 males and 7 females; the ages ranged from 51-75 years (median, 63). In ten patients the histological diagnosis was well-differentiated adenocarcinoma. Four patients had moderately differentiated adenocarcinoma, and in seven patients the histology was undifferentiated carcinoma. Patients received between two and 21 courses of COMF (mean, 8.4). Follow-up lasted between 2 and 58 months (mean, 18.7).

Table 3 summarizes the response rates and their duration. CR and PR were observed in ten patients (47.6%). Seven patients (33.4%) showed no change and in four patients (19%), the disease progressed. The median duration of response in the CR and PR patients was 43 and 8 months, respectively.

Table 4 summarizes toxic effects during therapy. Toxicity occurred in 14 patients (67%). Leukopenia was observed in three patients (14.3%) with a nadir of 1900 cells/mm^3. Thrombocytopenia was found in only one patient and the lowest count was 88,000 cells/mm^3. All signs and symptoms of toxicity were reversible and only transient discontinuation or dose modification was necessary.

DISCUSSION

The controversy in the treatment of metastatic carcinoma of unknown origin is yet unsolved. Various authors have dealt in recent years with the diagnostic

TABLE 2.—Site of metastatic disease and patient data

Patient No.	Site of histological diagnosis*	Sex	Age (yrs)	Performance status (%)
1	Left neck (LN)	F	68	80
2	Bone	M	60	60
3	Left supraclavicular (LN)	M	70	70
4	Bone	M	70	80
5	Right inguinal (LN)	M	61	100
6	Left supraclavicular (LN)	F	68	70
7	Right axilla (LN)	M	63	100
8	Left supraclavicular (LN)	F	55	50
9	Bone	M	68	60
10	Left supraclavicular (LN)	M	69	50
11	Left inguinal (LN)	M	57	80
12	Brain and right inguinal (LN)	M	67	90
13	Brain and lung	M	61	50
14	Brain and right axilla (LN)	M	63	70
15	Bone	F	64	70
16	Right inguinal (LN)	F	75	80
17	Brain	M	70	50
18	Brain	F	51	80
19	Brain, liver, supraclavicular (LN)	F	61	60
20	Left neck (LN)	M	60	70
21	Liver and left neck (LN)	M	60	70

*LN = lymph node.

TABLE 3.—Response rate and duration

Response*	No. of patients (%)	Duration (mos)		
		Range	Mean	Median
CR	6 (28.6)	10–58	36.5	43
PR	4 (19.0)	5–10	7.7	8
NC	7 (33.4)	3–18	10.8	12
P	4 (19.0)	0	0	0

*NC = no change; P = disease progression.

aspects of ACUP (1,2,4–6). Little, however, has been added to the therapy of this entity. A major difficulty in the chemotherapy of ACUP is drug selection, which usually has to be based on the possible primary origin of the tumor. Theoretically, one should strive for a broad range of drug activities in the treatment of ACUP tumors. A wide range of chemotherapeutic activity is essential from the cell kinetic and metabolic or phenotypic points of view. It was shown previously that most ACUP tumors arise from regions either above the diaphragm, eg, lung and breast, or below the diaphragm, eg, pancreas (5). Although we carried out all relevant investigations to exclude treatable tumors such as breast, the final primary site remained unknown. A microscopic primary tumor cannot be excluded entirely. This remains a pitfall in this entity of ACUP. However, as patients presenting with metastatic disease need to be treated, the different possibilities have to be taken into consideration when drugs are selected. COMF includes four drugs which proved to have some activity in these tumor sites whether administered as single drugs or in combination regimens (3). COMF was, therefore, selected to treat all patients presented to our department during the years 1979–1985 in whom all investigations failed to reveal the location of the primary tumor site.

TABLE 4.—Toxic effects during therapy

	No. of patients	%
Nausea	5	23.8
Vomiting	6	28.6
Diarrhea	6	28.6
Alopecia	7	33.3
Weakness	3	14.3
Peripheral neuropathy	3	14.3
Stomatitis	2	9.5
Leukopenia	3	14.3
Thrombocytopenia	1	4.8

The overall response rate (CR plus PR) in our group was 47.6%. Sixty percent of the responders had metastatic disease in lymph nodes. Of the ten responders, only one PR was observed in a patient with brain, liver, and lymph node involvement (Patient 19). All other responders belonged to the lymph node involvement category. The location of the involved lymph nodes in these patients was above the diaphragm in six patients, and below the diaphragm in four patients. Other reports in the literature showed responses varying from 5%–50% (6–12).

The COMF regimen in the present group of patients showed only minor toxicity. Slight and transient myelosuppression, nausea, and vomiting were seen, none of which was life threatening or caused definite discontinuation of treatment. The COMF combination seems to serve as broad-spectrum chemotherapy, with some level of activity in ACUP; it is tolerable to the patients and should be advocated, therefore, for further clinical study.

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Cardiotoxicity in Patients Treated With Mitoxantrone: Southwest Oncology Group Phase II Studies[1,2]

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A model describing the development of cardiotoxicity as a function of cumulative dose of a drug and other covariates is presented. Methods are given for testing and illustrating a cumulative dose effect. Also, equations for estimation of the probability of developing cardiotoxicity in the presence or absence of competing risks are given. The methods are illustrated for mitoxantrone by means of data obtained from Southwest Oncology Group studies. [Cancer Treat Rep 71:609-613, 1987]

Clark et al (1) described the cardiotoxic response [congestive heart failure (CHF) or decreased ejection fraction (DEF)] associated with cumulative mitoxantrone dose in patients entered in phase II protocols. Proportional hazard regression was used to establish that there was a significant association between cumulative dose and cardiotoxicity, but the dose-response relationship was characterized by means of a Kaplan-Meier curve (2) using final cumulative mitoxantrone dose in place of time to event. This method of representing the dose-response relationship, however, confounds a time effect, if one exists, with dose. For example, even when there are no dose-response effects, the method will produce one because the duration of exposure to the background risk of cardiotoxicity is greater for patients who have survived longer to receive higher cumulative dose levels. Further, when there are dose and time effects, the method would tend to underestimate risk at low dose levels because of short survival times and overestimate risk at high dose levels because of longer survival times. The purpose of this paper is to present methods (*a*) to determine if a dose-response relationship exists; (*b*) to estimate the probability of developing cardiotoxicity as a function of dose, time, and other covariates; and (*c*) to show how these relationships may be illustrated. Conceptually, our method estimates the effect of dose on instantaneous risk of cardiotoxicity in the following way: for each time a toxic event occurs, the technique compares the cumulative dose of the drug for patients with a toxic event at that time to the cumulative dose of patients who are alive at that time and have not had a toxic event. These comparisons are then summed over each time of a toxic event. This method corrects for the difference in cumulative dose between patients with toxicity and other patients that is simply due to the difference in the length of follow-up.

MATERIALS AND METHODS

Information on mitoxantrone administration, cardiotoxicity, and selected patient characteristics was collected for 801 patients entered in 14 phase II protocols of mitoxantrone performed by the Southwest Oncology Group. Mitoxantrone was administered by iv infusion over 30 minutes in 150 ml of 5% dextrose in water. An initial dose of 10–14 mg/m^2 was repeated every 3 weeks in all but one protocol, leukemia, which used a 5 × daily dose schedule. Modifications of dose schedules were based on nadir wbc counts. Cardiotoxicity was determined retrospectively: either CHF (12 patients) or DEF (12 patients). Further details of the patient population may be found in reference 1.

Statistical Methods

The proportional hazards model (3,4) was used to relate the hazard of toxicity (j = 1) or death (j = 2) to the covariates through the model

$$h_j(t) = h_{0j}(t) \exp(b_{1j}Z_1(t) + b_{2j}Z_2 \ldots b_{kj}Z_k), \quad [1]$$

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where $h_j(t)$ is the hazard at time t, $h_{oj}(t)$ is the underlying hazard at time t, $Z_1(t)$ is the cumulative mitoxantrone dose at time t (a time-dependent covariate), $Z_2 \ldots Z_k$ are non-time-dependent covariates (age, sex, prior mediastinal irradiation, and prior doxorubicin dose), and b_{ij} is the regression coefficient for the ith covariate and the jth outcome to be estimated from the data by means of BMDP2L (5)

Cumulative mitoxantrone dose, a time-dependent covariate, was summarized as a linearly increasing dose to final cumulative dose on the final dosing day. Cumulative doses at times between initial and final doses were obtained by interpolation. Selection of covariates and estimation of their regression coefficients were based on backward elimination of variables whose contributions, based on the log-likelihood statistic, were not significant ($P > 0.05$) (5). The retention of cumulative dose of mitoxantrone after elimination of nonsignificant variables and controlling for those remaining was considered an indication of a significant dose-response relationship. General methods of variable and model selection are described (6). Regression coefficients relating covariates to a specific outcome may be estimated by considering outcomes other than the one of interest as censored (4), provided that the outcomes can be considered independent and the occurrence of one outcome does not imply the occurrence of another or "give information" about another. The latter condition is called "independent noninformative censoring." An example of informative censoring might be when toxicity is always followed by death. Thus, censoring of toxic events would interfere with the estimation of the death rates. This is not the case here, since all toxic events preceded death by a considerable time period (1). Thus, regression coefficients for the toxicity outcome were determined with death as a censored observation. Alternatively, when death was the outcome of interest, toxicity was considered as a censoring mechanism. Cumulative dose of mitoxantrone was not included in the model with death as the outcome because the dosing was discontinued at progression of disease. Data were insufficient to estimate the effects of the large number of sites together with other covariates in a backward elimination model; therefore, the role of site of disease in development of cardiotoxicity was examined by means of forward stepwise regression. Dummy variables represented each site, with breast and gastric cancer forming the background sites for comparison (5).

The assumption of proportional hazards was tested by means of the addition of dose × time interaction terms in the model and graphically by examining the partial residuals of the covariates (7).

For a patient with covariate values $Z_2, \ldots, Z_k$ who receives a planned cumulative dose specified by function D(t), the net probability of developing toxicity as a function of time is estimated from the following equation:

$$P(t) = 1 - \exp\left\{ -\sum_{i:t_i \le t} h_{oli} \exp(b_{11} D(t_i) + b_{12} Z_2 \ldots b_{1k} Z_k) \right\}, \quad [2]$$

where

$$h_{oji} = \frac{m_{ij}}{\sum_{r \in R_i} \exp\left(\sum_k b_{jk} Z_k^r(t_i) \right)} \quad [3]$$

is an estimate of the hazard of risk j concentrated at the failure time t_i (8), m_{ij} is the number of events of type j at t_i, R_i is the risk set at t_i, r is a member of R_i, and Z_k^r is the value of covariate k for individual r. The summation in Equation 2 is over all times t_i at which a toxic event occurs. D(t) is an external time-dependent covariate in the sense of Kalbfleisch and Prentice (4). Three cumulative dose functions D(t) were studied representing typically low, moderate, and high dose schedules of mitoxantrone, ie, 24 mg/m^2 by 60 days, 60 mg/m^2 by 150 days, and 120 mg/m^2 by 300 days. The "net" probability of developing toxicity given by Equation 2 represents the probability of developing toxicity if the competing risk of death from noncardiotoxic causes were removed.

The crude probability of being alive and developing toxicity is estimated for the same dose schedule and times of toxicity from the following equation:

$$S(t) = \sum_{t_i \le t} h_{o1i} \exp(f_{1i}) \exp\left\{ -\sum_{j=1}^{2} \sum_{t_m < t_i} h_{ojm} \exp(f_{jm}) \right\}, \quad [4]$$

where h_{oji} is an estimate of the underlying hazard for risk j: toxic event (j = 1) and death (j = 2) at failure time t_i as computed in Equation 3, and

$$f_{ji} = b_{j1} D(t_i) + b_{j2} Z_2 + \ldots b_{jk} Z_k.$$

Equation 4 represents the probability of a patient living long enough to have a toxic event at time x integrated for all $x \le t$ (9). The first summation in Equation 4 is over all times t_i at which a toxic event occurs. The final summation is over this same set of times for j = 1 and over the set of times at which deaths occur for j = 2. The "crude" probability of developing toxicity by time t given by Equation 4 differs from the net probability for it includes the requirement of actually surviving long enough for the toxic potential of the treatment to actually manifest itself.

Confidence intervals were computed by methods developed by Mather et al.[6]

A FORTRAN computer program was written to calculate the net and crude probabilities of developing cardiotoxicity and the variance of the net probabilities. Input consists of (*a*) toxicity time, mortality time, and covariates for each patient; (*b*) regression coefficients and variance-covariance matrix from the proportional hazards model; and (*c*) the planned cumulative dose functions. An example follows illustrating the methods for mitoxantrone cardiotoxicity.

RESULTS

The proportional hazards regression identified cumulative mitoxantrone dose and prior doxorubicin dose as

[6] MATHER FJ, SIMON RM, CLARK GM, et al. A method for the evaluation of dose-toxicity relationships in clinical trials. Manuscript in preparation.

TABLE 1.—Regression coefficients and SEs for time-dependent covariate model for cardiotoxicity (CHF or DEF) and mortality*

Variable	Toxicity	Death
Mitoxantrone (cumulative dose)†	0.0275 ± 0.0058	‡
Doxorubicin (cumulative dose)	0.0017 ± 0.0008	NS
Mediastinal irradiation§	NS	0.4232 ± 0.1259
Age (yrs)	NS	NS
Sex	NS	NS

*NS = eliminated from consideration on the basis of log-likelihood test.

†Time-dependent covariate.

‡Cumulative dose of mitoxantrone was omitted from this model because dose was discontinued at progression of disease.

§Coded: 0 = no; 1 = yes.

prognostic variables significantly associated with increased cardiotoxicity (table 1). Prior mediastinal irradiation, age, and sex were not helpful in predicting cardiotoxicity. The model was re-evaluated with only CHF as an outcome to assess the effect of DEF. The results were nearly identical.

Only mediastinal irradiation was significantly associated with increasing hazard of death. There were 14 disease sites and at one of these sites (leukemia) there were two dose schedules. Only head and neck as a site showed a tendency to increase the hazard of toxicity, and, after adjustment for multiple comparisons, the contribution was not considered significant. No deviations from the proportional hazards model were detected.

The net probability of developing cardiotoxicity by a given time for three dose schedules is shown in figure 1. The dose schedules are based on a linearly increasing function of dose in time of 3 mg/m^2/week to a total of 24, 60, and 120 mg/m^2. The curves are drawn to 442 days because that is the latest time at which a toxic event occurred. The figure indicates that by Day 442, in the absence of death, about 4% of the population on a low-dose schedule is expected to develop cardiotoxicity, 6% of the medium-dose group, and 15% of the high-dose group. An average prior dose of doxorubicin of 134.2 mg/m^2 was used in this calculation. The net probabilities are hypothetical, however, because the true risks may depend on how other causes of death are removed.

The crude probability of being alive and developing toxicity is shown in figure 2. The crude probabilities are less than the net probabilities (fig 1) because of the high mortality due to disease in this population. The dose schedules are the same as for the net probabilities (fig 1); a prior doxorubicin dose of 134.2 mg/m^2 and no prior mediastinal irradiation are assumed. The crude probabilities of developing cardiotoxicity by 442 days for the three dose schedules are 1.5%, 2.8%, and 5.4%.

The dose-response relationship has been shown in previous literature (1) by means of a Kaplan-Meier survival curve applied to final cumulative mitoxantrone dose rather than time to toxic event. We compare this method with the net probability of developing cardiotoxicity within 442 days for dose schedules resulting in final cumulative doses of 24, 40, 50, 60, 90, 100, 120, 150, 160, and 189 mg/m^2 (accumulating at 3 mg/m^2/week). The net probability curves are plotted versus final cumulative dose for three levels of prior doxorubicin dose: none and 134.2 and 250 mg/m^2 (figs 3 and 4). The Kaplan-Meier estimate tends to underestimate the net probability of developing cardiotoxicity at low doses and overestimate at higher doses (fig 3). This pattern is similar in the more homogeneous group with no prior doxorubicin (n = 285) (fig 4).

DISCUSSION

Evaluation of the relationship of chronic toxic events to cumulative dose is difficult. The toxic events are often rare, necessitating large sample sizes, surveillance is often imperfect, background incidence rate may change over time, and there is sometimes heavy censoring due to patient deaths and evidence of toxicity may be delayed. The methods of this paper provide a framework for trying to deal with some of these problems. Inadequate surveillance cannot, however, be corrected by statistical analysis and hence these methods are best suited to the study of toxic events that are easily monitored. The diagnosis of CHF is readily made; however, diagnosis of DEF may depend on the number of ECGs performed.

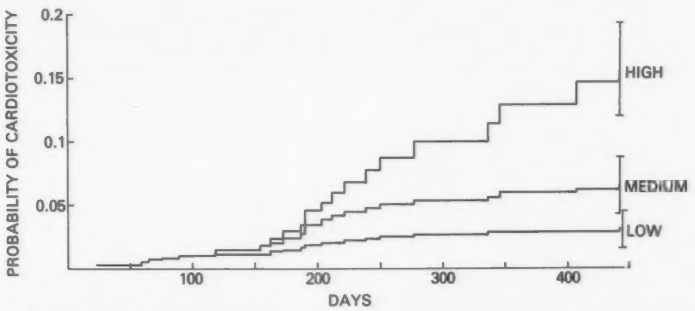

FIGURE 1.—Net probability of developing cardiotoxicity (CHF or DEF) by time since treatment began. 3 dose schedules are illustrated where final cumulative dose of mitoxantrone is as follows: low, 24 mg/m^2 by 60 days; medium, 60 mg/m^2 by 150 days; and high, 120 mg/m^2 by 300 days. Interval shown at 442 days is ± 1 SE.

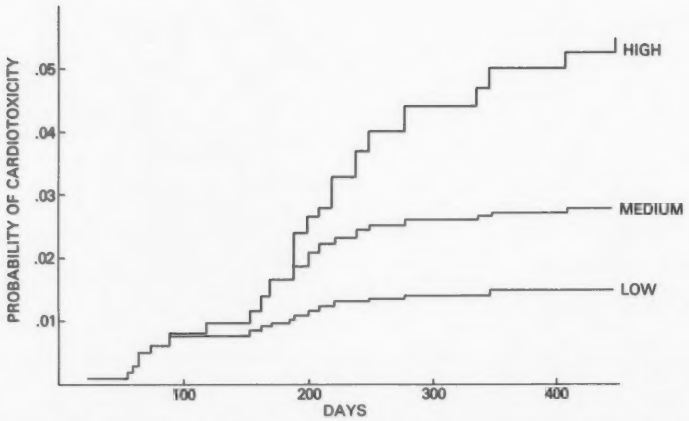

FIGURE 2.—Crude probability of developing cardiotoxicity (CHF or DEF) by time since treatment began. 3 dose schedules are illustrated where final cumulative dose of mitoxantrone is as follows: low, 24 mg/m^2 by 60 days; medium, 60 mg/m^2 by 150 days; and high, 120 mg/m^2 by 300 days.

Sufficient variation in time of cumulative dose must be present so that the effects of dose and time may be separated. We have implicitly assumed that unmodeled factors leading to the variation of dose in time, ie, modification of dose for acute toxicity or discontinuation of dose for progression of disease, are not in themselves predictors of cardiotoxicity. This is a necessary assumption for the analysis of an observational database. We see no reason to suspect this assumption but it may not be true. Planning trials to have different cumulative doses for similar patients would assure the separation of dose and time effects.

A check of the proportional hazards assumption by the addition of terms in dose and time and by examining the residuals as suggested by Schoenfeld (7) revealed no detectable deviation. The power of detecting such deviations, however, is not large, considering there are only 24 toxic events. The predictive accuracy of this model must be verified in a separate population.

The model is general enough to study the delayed development of toxicity in which there is a lagtime, l, between the dose attained and appearance of toxicity. The dose effect under such a model is studied by using the dose attained l time units prior to the time, t, for $t > l$ as the time-dependent dose covariate and 0 otherwise. Further, there is the possibility of a "waning effect" in which cumulative dose begins to lose its effect to develop toxicity in the time period subsequent to attaining a final dose. For example, doses at times t, subsequent to the final dose, d, at time t*, may be modeled as dexp (−b(t−t*)), when b is chosen to represent the type of decay desired.

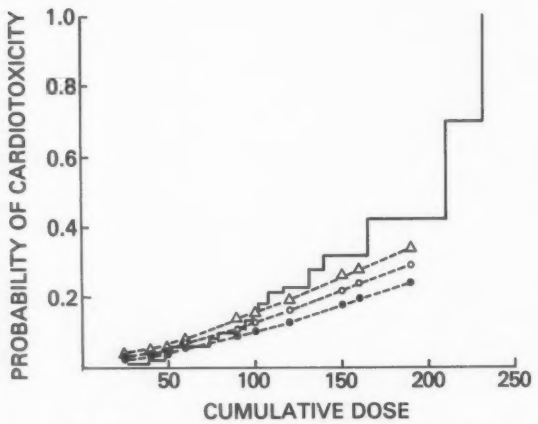

FIGURE 3.—Probability of cardiotoxicity (CHF or DEF) vs dose. Estimation by (*a*) Kaplan-Meier (——), and (*b*) net probability by 442 days by prior doxorubicin dose: none (●--●); 134.2 mg/m^2 (○--○); and 250 mg/m^2 (△--△).

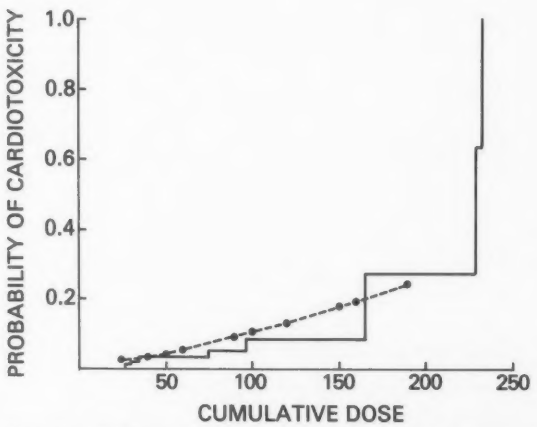

FIGURE 4.—Probability of cardiotoxicity (CHF or DEF) vs dose among those with no prior doxorubicin. Estimation by (*a*) Kaplan-Meier (——), and (*b*) net probability by 442 days (●--●).

The methods described in this paper may be used to study the relative toxicity of two drugs. Plots of the net probability of developing toxicity by time t for fixed covariates and dose schedule for each drug will illustrate differences in toxicity between two drugs. Alternatively, for various dose schedules and selected covariate values, the net probability of developing toxicity in a fixed time period may be plotted for each drug to illustrate differences in toxicity. Plots of net probabilities adjust for differences between groups in the level of other competing risks, in this case, causes of death.

Previous literature generally uses net probability to express the risk of developing toxicity; however, the crude probability may, in some cases, be more appropriate. In this study the crude probability gives the risk of developing cardiotoxicity and being alive. Although the risk of survival was determined from this study together with covariates affecting that risk, another survival curve from some other population could have been used as a standard with which to compute the crude probabilities.

The determination of safe operating ranges is a matter of balancing the risk of toxicity against the benefit of decreased mortality or morbidity. For this purpose, the net curve illustrates the maximum risk of developing toxicity for a given dose schedule if there were no removal from other risks. The risk of actually developing toxicity, of course, decreases as the risk of removal from other causes increases.

Some explanation is in order concerning the interpretation of the Kaplan-Meier curve based on dose. Even in the absence of time and dose effects the method produces an apparent dose-toxicity relationship because the duration of exposure to background risks of cardiotoxicity is greater for patients who survived long enough to receive the higher cumulative dose levels. If, however, the background rate of development of toxicity is low, the follow-up time is relatively short, and there is a strong dose effect, then it may give a close approximation. In general, the Kaplan-Meier curve would tend to underestimate risk at low-dose levels (because of shorter exposure times) and to overestimate the risk at high-dose levels (because of longer exposure times). It is for this reason that we recommend that the identification of maximum tolerable dose be explored through the plotting of net probabilities of different dose schedules through time rather than by examining a Kaplan-Meier curve or equivalent analysis based only on dose and ignoring time to event. Net probability curves have been estimated as far as the longest toxicity time, 442 days, which is all that the data allow.

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Pilot Trial of Prolonged Continuous-Infusion 5-Fluorouracil and Weekly Cisplatin in Advanced Colorectal Cancer[1]

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Thirty-five patients with previously untreated, advanced, measurable metastatic colorectal carcinoma were treated with a 12-week course of continuous 5-fluorouracil (5-FU) and weekly cisplatin. Twenty of 32 evaluable patients responded (five complete and 15 partial responses), for an overall response rate of 63% (90% confidence limits, 43%–75%). Toxicity was generally mild and reversible and included mucositis (40%), painful erythema of the palmar and plantar skin (30%), diarrhea (21%), nausea and vomiting (15%), and leukopenia (6%). One patient died of sepsis secondary to mucositis and myelosuppression. This program is a well-tolerated outpatient regimen for metastatic colorectal carcinoma. The response rate is higher than expected for 5-FU and cisplatin and suggests clinical therapeutic synergism at this dose rate and schedule. [Cancer Treat Rep 71:615–618, 1987]

Recent clinical trials of colorectal cancer have attempted to improve the effectiveness of currently available chemotherapeutic agents by manipulating dose and schedule. 5-Fluorouracil (5-FU) is the most active chemotherapeutic agent in colorectal cancer, producing objective responses at rates of 5%–25% when given in a weekly iv bolus schedule (1). Drug scheduling appears to result in improved efficacy; daily iv bolus loading or brief continuous iv infusion schedules improve response rates by 10%–15% (2–4) and prolonged continuous infusions for periods of ≥ 3 months have been well-tolerated and have produced response rates of 31%–39% (5–9). This apparent effect of prolonged infusion has been demonstrated in vitro in a variety of human colorectal carcinoma lines in which increasing the duration of exposure to 5-FU was more important for cell killing than increasing the concentration (10).

The strategy of combining 5-FU with other chemotherapeutic drugs has not led to meaningful improvements in treatment results (11). Most trials have combined these drugs with 5-FU given as a bolus or brief infusion, however, and there are few data on the effectiveness of prolonged infusions of 5-FU combined with other chemotherapeutic agents.

Although early clinical trials of cisplatin demonstrated a low response rate in patients with advanced colorectal carcinoma (12–14), in vitro studies have shown it to be an efficient agent against an established human colon carcinoma cell line (15). Additive or synergistic antitumor effects were seen when cisplatin was combined with 5-FU in preclinical studies (16,17). The combination of 5-FU and cisplatin has been found to be clinically active in lung carcinoma, esophageal carcinoma, and head and neck cancers (18–20). Experience in colorectal cancer is limited but the results of some clinical trials suggest synergism (21–23).

Based on these observations we designed a regimen that would provide protracted exposure to 5-FU and cisplatin and evaluated the efficacy and toxicity of the combination in patients with metastatic colorectal carcinoma (24).

PATIENTS AND METHODS

Patients were considered eligible for study if they had bidimensionally measurable metastatic carcinoma of the colon or rectum, performance status [Cancer and Leukemia Group B (CALGB)] of 3 or less, and no prior chemotherapy. Patients were excluded from this trial if there was pre-existent renal dysfunction (creatinine > 1.5 mg/dl). Written informed consent was required of all patients prior to initiation of therapy.

Treatment consisted of continuous-infusion 5-FU at a daily dose of 300 mg/m^2 and a weekly infusion of cisplatin at a dose of 20 mg/m^2. The 5-FU was delivered by a Travenol or Cormed pump via an indwelling subclavian venous catheter. The cisplatin was infused over 20 minutes with 1 hour of pre- and post-treatment hydration and furosemide at 20 mg 5 minutes prior to cisplatin. Patients received individualized antiemetic

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regimens consisting of dexamethasone, diphenhydramine, and droperidol or metoclopramide. Therapy was continued for 12 consecutive weeks or until tumor progression intervened. All patients were re-evaluated for response after 12 weeks. Those patients achieving complete or partial response were maintained on continuous 5-FU and twice-monthly cisplatin until disease progression or prohibitive toxicity developed. 5-FU was interrupted when mucositis, diarrhea, or painful palmar or plantar erythema developed and was resumed at 50% dose when toxicity resolved. Subsequent escalations to 75% were undertaken whenever possible.

All patients underwent re-evaluation for response at 12 weeks and earlier if indicated. Complete response was defined as when symptoms and signs related to the tumor resolved and when radiologic abnormalities normalized. Partial response was defined as a reduction of at least 50% in the sum of the products of all measurable lesions. Hepatic tumor measurements were determined by computerized axial tomography; changes in physical examination dimensions of hepatic size were not considered sufficient to qualify as a response. Stable disease was defined as either a $< 50\%$ reduction or a $< 25\%$ increase in tumor size during the 12 weeks of therapy. Progressive disease was defined as a $> 25\%$ increase in the size of measurable tumor mass or the appearance of any new lesions. Time to disease progression and survival were calculated from the first day of treatment.

Before beginning treatment and at the time of response evaluation, each patient underwent full clinical and laboratory assessment including history, physical examination, measurement of lesions, carcinoembryonic antigen (CEA), cbc, complete chemistry profile, chest x-ray, and computerized tomographic scan of the liver. Clinical toxicity, blood cell counts, and creatinine values were evaluated weekly.

RESULTS

From September 1, 1984, to October 1, 1985, 35 patients met eligibility criteria and were entered in the trial. Three patients were inevaluable for response: one patient died of a pulmonary embolus on Week 3, one developed normal-pressure hydrocephalus and dementia necessitating removal from study on Week 3, and one who clinically benefited from the treatment was excluded because his pretreatment disease was not accurately measurable. The pretreatment characteristics of the 32 evaluable patients are shown in table 1. The majority of patients were symptomatic (74%) and 13 of 35 (37%) had a CALGB performance status of 2 or greater. Sites of measurable disease were liver in 27 patients, lung in three patients, abdomen or pelvis in ten patients, and more than one site in five patients. Most patients had pretreatment laboratory parameters associated with a poor prognosis; an elevated alkaline phosphatase or LDH was present in 21 of 33 evaluable patients and 12 of 28 patients had a CEA > 500 ng/ml (5,6).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 65 (37-81)	
Male/female	19/16
Performance status (CALGB)	
0	9
1	13
2	8
3	5
Sites of disease	
Liver	27
Abdomen-pelvis	10
Lung	3
Other	2
Laboratory	
Median alkaline phosphatase in mIU/L (range): 212 (50-1048)	
Median LDH in mIU/L (range): 312 (66-2830)	
Median CEA in ng/ml (range): 302 (1-9860)	

Five patients achieved complete response (16%) and 15 patients achieved partial response (47%), for an overall objective response rate of 63% (table 2). Seven patients had stable disease and three had disease progression prior to completion of 12 weeks of therapy. Among complete responders, Patients 1 and 2 had a $> 50\%$ involvement of liver with tumor, Patient 3 had a 4×6-cm abdominal mass growing through the abdominal wall, and Patients 4 and 5 had small volumes of meta-

TABLE 2.—Response (32 evaluable patients)

Response	No. of patients (%)	No. with disease progression	Duration of response (wks)*	
			Median	Range
Complete	5 (16)	4	30	20-92†
Partial	15 (47)	15	24	15-52
Stable disease	7 (21)	7	27	16-44
Disease progression	5 (16)	5	9	8-12
Overall	20 (63)	19	30	15-92†

*Measured from initiation of therapy to time of disease progression.
†90% confidence limits, 43%-75%.

TABLE 3.—Toxicity (34 evaluable patients)

Toxic effect	No. of patients (%)
Anorexia, altered taste	34 (100)
Mucositis	15 (44)
Hand-foot syndrome	10 (30)
Diarrhea	7 (21)
Nausea, vomiting	5 (15)
Myelosuppression	2 (6)

static disease. All five complete responders had an elevated pretreatment CEA that decreased approximately fourfold to fiftyfold after treatment but only one became normal. A pretreatment LDH and alkaline phosphatase were increased in three of these patients and completely normalized after treatment. Among the ten partial responders and five patients with stable disease who had elevated pretreatment CEAs, all decreased by > 50% after 12 weeks of treatment. Four of the complete responders and all 15 partial responders have subsequently progressed in 15–52 weeks. One patient remains in complete response after 92 weeks. The median duration of response is 30 weeks. Median survival is 50 weeks (range, 14–92).

Toxicity was generally mild and reversible (table 3). The one inevaluable patient died of a pulmonary embolus 3 weeks into therapy without apparent toxicity. Six patients had no toxicity, but in 23, toxicity did require a reduction in dose during the 12-week course of treatment. Ulceration of the oral mucosa was mild in nine patients, moderate in three, and severe (required iv fluids) in two. Anorexia and altered taste sensation were encountered in all patients. Five patients had mild nausea and seven had diarrhea. Erythema, tenderness, and peeling of the palmar and plantar skin developed in ten patients (30%) and usually subsided with interruption of the 5-FU infusion for 1–2 weeks. Leukopenia was encountered in two patients. One patient developed gram-negative sepsis with a wbc count of 3900/μl and subsequently recovered fully. The other patient presented with severe mucositis and leukopenia (wbc count, 1100/μl, with 70% neutrophils on the differential) on Week 15 and died of sepsis. Thrombocytopenia was not encountered. There were no instances of nephrotoxicity (serum creatinine > 2.0 mg) or clinically apparent loss of hearing or alopecia.

DISCUSSION

Considering the modest therapeutic activity of 5-FU and cisplatin as single agents in colon carcinoma, the response rate observed in this trial is higher than expected. We were not able to identify a selection bias to explain the high number of objective responders. Pretreatment characteristics indicate that this series of patients had many factors known to be associated with a poor prognosis (table 1) (25,26).

It is unlikely that the high response rate of this combination is due to a pharmacokinetic effect of cisplatin on the metabolic handling of 5-FU. If this interaction was responsible, one would expect to see some quantitative or qualitative change in the clinical toxicity; however, the toxicity observed was similar to that described for the individual drugs when used separately (5,27). The intensity of the therapy may have been responsible for some increase in therapeutic effect, since the protocol design assured that each patient was treated to the point of dose-limiting toxicity.

The clinical activity of cisplatin as a single agent in untreated colorectal carcinoma has not been defined. Early studies found no response in previously treated patients (13,14). A response rate of 70% was noted when intra-arterial hepatic infusions were given to 30 patients with previously untreated colorectal cancer (28). There have been no trials reported of systemic venous infusions of cisplatin to previously untreated patients.

Previous trials of these drugs in combination for treatment of advanced colorectal carcinoma have shown a wide range of antitumor activity (table 4). These trials

TABLE 4.—Summary of clinical trial results

Clinical trial	Ref No.	Regimen*	No. of patients†	Response rate (%)
Cantrell et al	Present study	5-FU by CIV × 12 wks; DDP iv every wk × 12	32 (0)	63
Loehrer et al	22	5-FU iv every wk; DDP iv every 3 wks	32 (0)	32
Madajewicz et al	29	5-FU by CIV × 120 hrs; DDP iv every day × 5	8 (8)	38
Shepard et al	30	5-FU by CIV × 120 hrs; DDP iv on Day 1	20 (7)	0
Muchmore et al	31	5-FU iv every day × 5; DDP iv on Day 1	17 (0)	0
Dy et al	23	5-FU by CIV × 120 hrs; DDP by CIV × 24 hrs	32	40

*CIV = continuous iv infusion; DDP = cisplatin.
†Values in parentheses = patients who had received prior chemotherapy.

have utilized 5-FU as a weekly bolus or 96-120-hour infusion and cisplatin every 3 or 4 weeks. The current study used a schedule of 5-FU delivered continuously for 12 weeks in conjunction with weekly cisplatin. It is possible that the cisplatin schedule is responsible for the higher response rate. Piver et al (27) have suggested that weekly cisplatin produces a higher response rate in patients with refractory ovarian carcinoma than high-dose intermittent cisplatin. Alternatively, an additive or synergistic interaction may be operating that depends on the protracted exposure to 5-FU and cisplatin. Confirmation of the findings of this study and elucidation of the dose and schedule factors involved must await future clinical trials.

Although we observed a high response rate, the duration of response was not prolonged. This may have been due to rapid development of biochemical resistance. Alternatively, tumor progression may have developed because of kinetic resistance since we reduced the frequency of cisplatin administration after the first 12 weeks of treatment. If this is true, an increase in the frequency or dose of cisplatin in future trials may lead to more prolonged disease control. The combination of continuous low-dose 5-FU with weekly cisplatin is a well-tolerated, effective regimen for patients with previously untreated metastatic colorectal carcinoma. Further trials of this regimen are warranted to confirm these observations and to explore the relationship of dose rate and response.

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Selective Enhancement by Menadiol of In Vitro Drug Activity in Human Lymphatic Neoplasms[1,2]

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The effect of menadiol (vitamin K_3) on fresh specimens of human lymphatic neoplasms (HLN) was tested by means of the differential staining cytotoxicity assay. Menadiol was tested alone and in combination with standard antineoplastic agents. Drug effects were then compared with the effects of the same drugs in normal human lymphocytes and in fresh specimens of human non-small cell lung cancer. By itself, menadiol was moderately toxic to HLN, but not to normal lymphocytes or non-small cell lung cancer. Menadiol, menadione, and two structurally related congeners were equitoxic to HLN cells, but sodium metabisulfite (present in menadiol solutions as a preservative) was nontoxic. Menadiol increased the cytotoxic effects of a number of standard agents in HLN but not in normal lymphocytes. Cell survival times with mechlorethamine, vincristine, and dexamethasone were converted from a range characteristic of drug resistance (ie, range observed in relapsed patients) to a range characteristic of drug sensitivity (ie, range observed in untreated patients) in the presence of menadiol. These effects occurred at a concentration (2.0 μg/ml; 4.7 μM) of menadiol which is probably clinically achievable and which did not deplete intracellular glutathione. Menadiol should receive clinical testing as a chemosensitizing agent in HLN. [Cancer Treat Rep 71:619-625, 1987]

Lymphatic neoplasms as a group are highly responsive to chemotherapy. Following the initial treatment, however, most patients eventually develop drug-resistant disease. If this acquired drug resistance could be circumvented, chemotherapy would be vastly improved.

Recently, we reported (1) that the differential staining cytotoxicity (DiSC) assay accurately discriminates between drug-sensitive and -resistant populations of fresh tumor cells obtained from patients with lymphatic neoplasms. Most important, we showed that cells from previously treated patients were significantly more resistant in the assay to the most important drugs than were cells from previously untreated patients. Additionally, metachronous assays performed on cells obtained from patients revealed increasing drug resistance when cells were repeatedly assayed after patients had received courses of intervening clinical chemotherapy. However, when repeated assays were performed in the absence of intervening chemotherapy, there was no significant change in the level of in vitro drug resistance.

These data indicate that the DiSC assay could be of value in studying agents with the potential for circumventing drug resistance. For example (1), the mean percent cell survival with vincristine in assays performed on cells from 16 previously untreated patients with acute lymphoblastic leukemia (ALL) was 25% ± 5.2% SEM. This assay range is, therefore, characteristic of a largely drug-sensitive cell population. However, the mean percent cell survival in assays on cells from 11 relapsed ALL patients was 65% ± 6.0% SEM. This assay range is, therefore, characteristic of a largely drug-resistant cell population. We postulate that active chemopotentiators could be identified by their ability to change the in vitro chemosensitivity pattern from a range associated with drug-resistant cell populations to a range associated with drug-sensitive cell populations. Furthermore, the relative selectivity of chemopotentiation could be studied by comparing assays performed on normal human lymphocytes with assays performed on neoplastic human lymphocytes.

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Menadiol (vitamin K_3) has been studied as a potential radiosensitization and chemosensitization agent (2). This agent is clinically available, inexpensive, and remarkably nontoxic (3). Menadiol has been proposed to sensitize cells to cytotoxic therapy by virtue of depleting intracellular glutathione or through the generation of toxic oxygen radicals (4). We now report the results of initial studies of this agent in a spectrum of human lymphatic neoplasms (HLN) and normal human lymphocytes.

METHODS

Patient Specimens

Specimens included EDTA-anticoagulated blood or marrow specimens and solid tumor biopsies obtained in accordance with the Human Studies guidelines of the collaborating institutions. In most cases, these specimens represented aliquots of material removed for unrelated indications and which were in excess of that required for diagnostic purposes. In cases where tissue was removed expressly for this study, prior IRB approval and informed consent in accordance with local institution and National Institutes of Health guidelines were obtained. Solid tumors were collected in complete tissue culture medium with antibiotics. Drugs were either obtained in powder form from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD (mechlorethamine and carmustine), or else were prepared by diluting vials of clinical drug solutions [menadiol (Synkavite, Roche), dexamethasone, and vincristine]. Structurally related analogs of menadione were provided by Dr. Alan Sartorelli of Yale University. Drugs were prepared at 10–100 times the desired final concentration and stored at −65°C until use.

Assay Methods

Specimens were assayed for chemosensitivity with the DiSC assay, as described elsewhere (1), which involves a 4-day continuous drug exposure in polypropylene tubes, staining dead cells with fast green/nigrosin, adding an internal standard of acetaldehyde-fixed duck rbcs, cytocentrifugation, counterstaining with Wright-Giemsa, and counting living neoplastic cells and duck red blood cells. The assay endpoint is the total mass of viable tumor cells following drug exposure, relative to control cultures without drug.

Glutathione Assay

Cells (5×10^6) were harvested and washed once in phosphate-buffered saline and pelleted by centrifugation. The supernatant was removed and cells were lysed in 1.5 ml of double-distilled water by vigorous mixing for 2–3 minutes, then stored at −65°C until analyzed (5).

Cell pellets were thawed and the glutathione level was determined by the method of Beutler et al (6). Glutathione levels are expressed as micrograms of glutathione per 10^7 cells.

RESULTS

Seventy-five specimens of HLN were studied. Most specimens were from patients who had previously received chemotherapy. These consisted of the following numbers of individual specimens: chronic lymphocytic leukemia (CLL), 57 specimens; ALL, seven; non-Hodgkin's lymphoma, six; and multiple myeloma, five. In addition, 39 specimens of normal peripheral blood lymphocytes were also studied. For comparison, we also evaluated six solid tumor specimens (non-small cell lung cancer).

Table 1 shows the activity of menadiol alone in normal lymphocytes and human neoplasms tested in the DiSC assay. These data show the average percent cell survival of the different categories of specimens following exposure to menadiol at 2.0 μg/ml for 4 days. Menadiol was by itself nontoxic to normal lymphocytes and solid tumor specimens, but was significantly toxic to lymphatic neoplasms.

Table 2 shows average percent cell survival times for normal lymphocytes and CLL cells exposed to a variety of antineoplastic agents, alone and in combination with menadiol (2.0 μg/ml). We attempted to study the potentiation of standard agents by menadiol and, conversely, the potentiation of menadiol by the same standard agents.

Standard agents (mechlorethamine, carmustine, vincristine, and dexamethasone) were mildly toxic to nor-

TABLE 1.—Sensitivity of normal lymphocytes and tumor cells to menadiol

Cell type	Average % cell survival ± SD in presence of menadiol (2.0 μg/ml)*	No. of specimens	*P* value†
Normal lymphocytes	103 ± 18	39	—
CLL	53.8 ± 35	57	< 0.0001
ALL	45.2 ± 22	7	< 0.0001
Multiple myeloma	64.2 ± 38	5	< 0.001
Non-Hodgkin's lymphoma	28.3 ± 32	6	< 0.0001
Non-small cell lung cancer	97.8 ± 20	6	Not significant

*% of control.
†Compared to normal lymphocytes.

TABLE 2.—Effects of menadiol on drug toxicity induced by standard antineoplastic agents on normal and neoplastic human lymphatic cells: % cell survival

Drug tested (μg/ml)*	Average % cell survival ± SD: Drug alone	Drug + menadiol (2.0 μg/ml)	P_1 value†	P_2 value‡
		Normal lymphocytes		
Saline (n = 39)	100	103.1 ± 18	> 0.25	—
Mechlorethamine (0.35)	70.5 ± 25	59.1 ± 22	> 0.05	< 0.01
Vincristine (0.08)	89.7 ± 25	84.5 ± 31	> 0.25	< 0.01
Dexamethasone (0.4)	80.6 ± 29	80.6 ± 24	> 0.25	< 0.01
		CLL		
Saline (n = 57)	100	53.8 ± 35	< 0.01	—
Mechlorethamine (0.35)	66.8 ± 32	30.6 ± 31	< 0.01	< 0.01
Vincristine (0.08)	54.8 ± 33	23.2 ± 27	< 0.01	< 0.01
Dexamethasone (0.4)	56.8 ± 29	26.4 ± 26	< 0.01	< 0.01
		ALL		
Saline (n = 7)	100	45.2 ± 22	< 0.01	—
Mechlorethamine (0.35)	73.5 ± 27	20.5 ± 8.1	< 0.01	< 0.05
Vincristine (0.08)	46.8 ± 26	18.8 ± 20	< 0.05	< 0.05
Dexamethasone (0.4)	58.0 ± 42	27.3 ± 24	< 0.05	< 0.10
		Multiple myeloma		
Saline (n = 5)	100	64.2 ± 38	< 0.01	—
Mechlorethamine (0.35)	41.5 ± 12	25.6 ± 15	< 0.05	< 0.05
Vincristine (0.08)	31.1 ± 25	28.7 ± 25	> 0.25	< 0.05
Dexamethasone (0.4)	52.1 ± 28	11.6 ± 8.0	< 0.01	< 0.05
		Non-Hodgkin's lymphoma		
Saline (n = 6)	100	28.3 ± 35	< 0.01	—
Mechlorethamine (0.35)	37.6 ± 38	10.0 ± 10	< 0.01	> 0.25
Vincristine (0.08)	38.2 ± 22	14.6 ± 20	< 0.05	> 0.25
Dexamethasone (0.4)	56.2 ± 29	39.0 ± 40	< 0.05	> 0.25

*n = No. of specimens.
†Significance of drug + menadiol results compared with drug-alone results.
‡Significance of drug + menadiol results compared with menadiol-alone results.

mal lymphocytes but were, in general, more toxic to CLL cells. Standard agents plus menadiol were not significantly ("P_1" column) more active than standard agents alone in normal lymphocytes, but standard agents plus menadiol were significantly more active than standard agents alone in CLL cells. Thus, chemotherapeutic enhancement by menadiol was relatively specific for CLL cells compared with normal lymphocytes. Analyzing the data conversely ("P_2" column), the standard agents plus menadiol were significantly more active than menadiol alone in both normal lymphocytes and CLL cells. By this analysis (and in the analysis shown in table 1), the cytotoxic effects of menadiol appeared to be more specific than those of the standard agents.

The effects of menadiol on drug toxic effects in other lymphatic neoplasms are shown in table 2. In general, menadiol was found to have significant effects in ALL, multiple myeloma, and non-Hodgkin's lymphoma, which were at least additive.

We also analyzed these results in a number of other ways. First, we restricted our analysis to those assays in which both standard antineoplastic agents alone and agents plus menadiol were tested. These results were then analyzed with a paired t test. In summary, for normal lymphocytes, there was no significant difference between standard drug alone and standard drug plus menadiol in the case of mechlorethamine, vincristine, dexamethasone, and carmustine. In contrast, in CLL menadiol significantly enhanced all of the above drugs. In ALL, menadiol significantly enhanced mechlorethamine and vincristine, but not dexamethasone. In multiple myeloma, menadiol significantly enhanced dexamethasone, but not vincristine and mechlorethamine. In non-Hodgkin's lymphoma, menadiol significantly enhanced vincristine, but not the other drugs (the above data are not shown).

Next, we analyzed the data qualitatively, as a function of whether or not specimens were sensitive (< 30% cell survival) or resistant (> 30% cell survival) to drugs in the presence or absence of menadiol. Table 3 shows that menadiol significantly enhanced mechlorethamine, carmustine, vincristine, and dexamethasone in CLL cells when analyzed by Fisher's exact test. In contrast, menadiol did not significantly enhance any of the above agents in normal peripheral blood lymphocytes.

We then proceeded to determine whether or not the sensitivity to menadiol (tested alone) of individual specimens was correlated with their sensitivity to the standard agents by plotting the in vitro sensitivity to menadiol alone as a function of in vitro sensitivity to

TABLE 3.—Effects of menadiol on drug toxicity induced by standard antineoplastic agents on normal and neoplastic human lymphatic cells in vitro: sensitive vs resistant*

Drug tested (μg/ml)	Drug alone		Drug + menadiol (2.0 μg/ml)		*P* value
	Sensitive	Resistant	Sensitive	Resistant	
		CLL			
Saline	0	57	18	39	< 0.0001
Mechlorethamine (1.17)	16	12	20	4	0.040
Mechlorethamine (0.35)	6	36	18	14	< 0.0001
Carmustine (3.3)	8	17	13	10	< 0.078
Vincristine (0.08)	12	32	20	9	< 0.005
Dexamethasone (0.4)	7	38	18	11	< 0.0001
		Normal lymphocytes			
Saline	0	39	0	39	1.0
Mechlorethamine (1.17)	8	3	9	0	0.14
Mechlorethamine (0.35)	2	23	2	18	0.61
Carmustine (3.3)	0	15	0	15	1.0
Vincristine (0.08)	0	22	1	18	0.46
Dexamethasone (0.4)	0	22	0	19	1.0

*Sensitive = < 30% cell survival; resistant = > 30% cell survival.

mechlorethamine alone. No correlation was seen (data not shown). Similar analyses with vincristine, dexamethasone, and carmustine also revealed no correlation between in vitro sensitivities to menadiol and the other individual drugs.

To determine whether the above effects of menadiol were related to glutathione content in the cells, glutathione levels were measured in CLL cells following incubation with menadiol. Menadiol caused only temporary minimal changes in glutathione levels, even in specimens highly sensitive to menadiol alone (eg, fig 1). After several days of continuous incubation with menadiol, a decreased content of glutathione was observed (data not shown). However, this diminution in glutathione content was probably a secondary consequence of menadiol-induced cytotoxicity rather than a primary event contributing to cytotoxicity, as suggested by the lack of major short-term effects of menadiol on intracellular glutathione content.

Finally, it should be noted that the above studies on

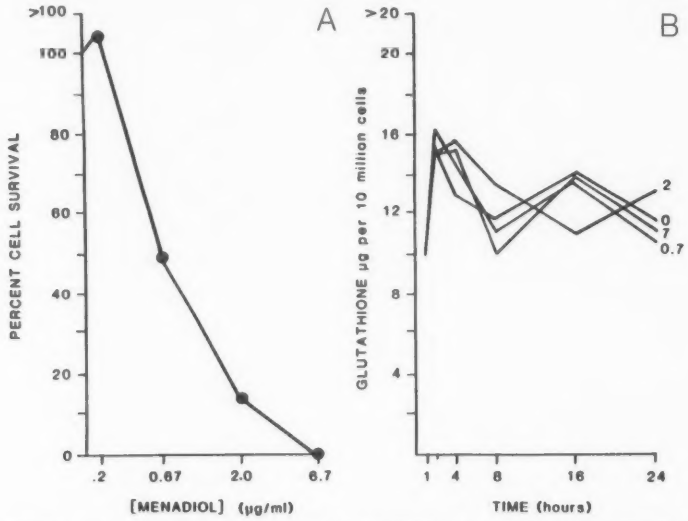

FIGURE 1.—A. Survival of CLL cells from a single patient after continuous 4-day exposure to graded concentrations of menadiol. These cells were highly sensitive to menadiol alone. B. Intracellular glutathione content of the same CLL cells during the first 24 hrs of exposure to the same concentrations of menadiol. Curves for 0, 0.67 (labeled 0.67), and 6.7 (labeled 7) μg/ml of menadiol are virtually superimposable at all times, and the curve for 2.0 μg/ml has only minimal variations.

TABLE 4.—Effects of Synkavite, menadione, sodium metabisulfite, and 2 menadione congeners tested against HLN in vitro

Diagnosis	Patient No.	% cell survival in DiSC assay: Synkavite (4.7 μM, 2.0 μg/ml)	Menadione (4.7 μM)	Sodium metabisulfite (1.0 μg/ml)	CMNQ (4.7 μM)	BCMNQ (4.7 μM)
CLL	1	0	0	99	0	0
CLL	2	0	0	73	0	0
CLL	3	3	5	103	44	3
CLL	4	4	2	81	42	2
CLL	5	11	19	97	1	1
CLL	6	13	97	102	44	0
CLL	7	34	42	108	2	29
CLL	8	66	88	87	51	46
CLL	9	91	98	91	50	105
CLL	10	94	86	75	100	93
CLL	11	98	77	89	98	76
Multiple myeloma	1	23	10	99	15	44
Multiple myeloma	2	57	37	108	2	29
Multiple myeloma	3	129	126	138	109	99
Values						
Mean		44.50	49.07	96.43	39.86	37.64
SD		44.07	44.61	16.42	39.43	40.35
SEM		12.22	12.37	4.55	10.94	11.19
P (vehicle vs drug)		< 0.001	< 0.001	> 0.5	< 0.001	< 0.001
P (Synkavite vs drug)			> 0.5	< 0.001	> 0.5	> 0.5

menadiol were carried out using the clinically approved Synkavite (Roche) preparation. This contains 0.5 mg of sodium metabisulfite as an antioxidant preservative for every 1.0 mg of menadiol. To determine the active moiety of the Synkavite preparation, we simultaneously tested (*a*) Synkavite [2.0 μg/ml (4.7 μM) of menadiol and 1.0 μg/ml of sodium metabisulfite], (*b*) menadione (4.7 μM), (*c*) sodium metabisulfite alone (1.0 μg/ml), and (*d* and *e*) two structural analogs of menadione (both at 4.7 μM). Menadione and its analogs require DMSO for solubilization, so that all of the above drugs (*a–e*) were tested in culture medium containing 0.25% dimethyl sulfoxide. Table 4 demonstrates that Synkavite, menadione, and the two menadione analogs (fig 2) were equitoxic to lymphatic neoplasms, while sodium metabisulfite was not toxic.

DISCUSSION

In previous investigations (1,8) we defined drug concentrations and percent cell survival times which were characteristic of drug-sensitive and -resistant populations of HLN studied in the DiSC assay. In the current paper we showed that administration of menadiol for 4 days in the concentration of 2.0 μg/ml (4.7 μM) frequently converted the cell survival times with mechlorethamine, vincristine, and dexamethasone from a range characteristic of drug resistance (ie, range observed in relapsed patients) to a range characteristic of drug sensitivity (ie, range observed in untreated patients) in the

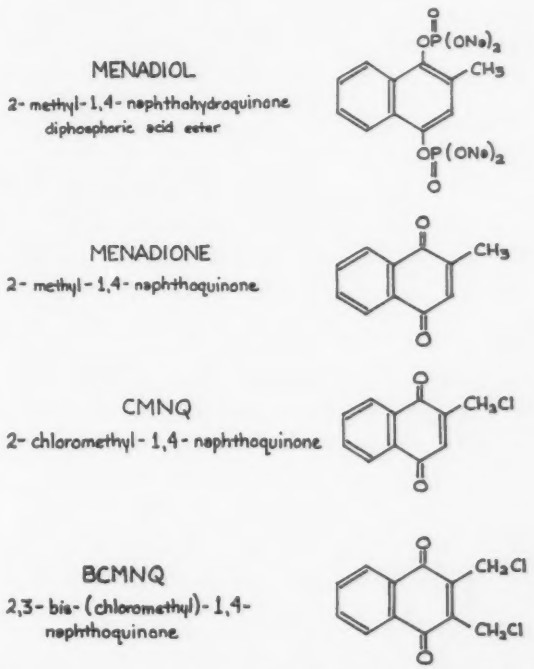

FIGURE 2.—Structures of menadiol, menadione, and menadione analogs synthesized by Lin and Sartorelli (7).

presence of menadiol. These effects were highly specific for lymphatic neoplasms when compared with normal human peripheral blood lymphocytes. Menadiol was, by itself, somewhat toxic to lymphatic neoplasms at this concentration, but was not toxic at the same concentration to normal lymphocytes or fresh specimens of human solid tumors. These data suggest that menadiol may have effects at least somewhat selective for neoplastic lymphoid cells.

Vitamin K has been studied clinically for > 40 years, since its identification as an antihemorrhagic agent (9). Menadiol has been suggested as a potential agent to sensitize tumors to radiotherapy (10), hyperthermia (11), and chemotherapy (12). The clinical results of menadiol as a radiopotentiator are somewhat conflicting (1). Chlebowski et al (13–15) have recently suggested the use of menadiol as a chemopotentiating agent. Based on the findings and conjectures of this group, a phase I clinical trial of menadiol is currently ongoing at the City of Hope Cancer Center, Duarte, CA (S. Akman, personal communication). Existing clinical data do indicate that menadiol is a remarkably well-tolerated and nontoxic agent (2). In one study, individual patients were reported to have received very high parenteral doses (≤ 1000 mg/day for 11 consecutive days) without serious toxicity (2). Other authors have shown that menadiol may suppress human neutrophil function in vitro at concentrations twofold to 20-fold higher than the concentration (2.0 μg/ml) tested in our studies (22). Existing but fragmentary pharmacokinetic data (2,16) would strongly suggest that the 2.0-μg/ml concentration range tested in vitro in the present communication is likely to be clinically achievable.

Menadiol by itself had in vitro activity in HLN. The mechanisms by which menadiol produces cytotoxicity and chemopotentiation are not known. Menadiol is rapidly converted in vivo to menadione (16) and the data in table 4 suggest that the active moiety in the Synkavite clinical solution is related to menadione (and not to the sodium metabisulfite preservative). Other related naphthoquinone analogs were comparably cytotoxic to menadiol and menadione (table 4). Menadione has been shown to perturb intracellular calcium flux (17,18) and calcium-dependent potassium flux (19). Menadione is capable of undergoing several further forms of chemical transformation within the cell. This intracellular transformation may generate reactive oxygen species and potentially deplete intracellular glutathione (2,4,20). The concentrations of menadiol required to deplete intracellular glutathione and to produce cytotoxicity in L1210 cells were reported by Akman et al (21) to be substantially higher (almost 10 times) than the concentrations studied by us. In our system, menadiol did occasionally produce a modest reduction (< 50%) in intracellular glutathione, which then rebounded to normal or supranormal levels within 24 hours. However, short-term glutathione reduction was not seen in other cases (fig 1) in which menadiol was profoundly cytotoxic. Thus, we do not feel that the effects of menadiol in this concentration range are likely to be due to changes in glutathione content. A more substantial and prolonged depletion of glutathione was observed only after several days of continuous exposure, and this was probably a secondary consequence of drug-induced cell death.

In summary, menadiol has the ability to enhance a number of standard cytotoxic agents in HLN freshly cultured in vitro. This enhancement appears to be selective, as determined by the relative lack of effects in normal lymphocytes at a comparable concentration. The concentration active against neoplastic cells is probably clinically achievable. Clinical trials are needed to determine maximally tolerated dosages and to provide evidence for clinical activity in HLN.

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Phase II Study of Mitoxantrone in Epithelial Ovarian Cancer[1]

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Forty-six patients with advanced epithelial ovarian cancer were treated with mitoxantrone (14 mg/m^2) given iv every 3 weeks. All patients had evaluable disease. Forty-one patients had had previous treatment, 38 with cisplatin. Twelve patients achieved partial response (two had had no previous treatment; ten had received previous treatment with cisplatin with or without other agents); two of the responders, both previously treated with cisplatin, achieved complete clinical remissions. Median duration of response was 24 weeks (range, 10-30). Toxicity was acceptable, with minimal subjective toxicity. Hematological toxicity was dose-limiting, but it was possible to increase the dose on one or more courses by 2 mg/m^2 in 16 patients. The drug was given as a bolus, and there was no evidence of acute cardiac side effects. These data suggest that mitoxantrone has significant activity in epithelial ovarian cancer and deserves further study. [Cancer Treat Rep 71:627-629, 1987]

Chemotherapy has a major role to play in the management of epithelial ovarian cancer (EOC), since most patients present with late-stage disease which is not amenable to complete surgical excision (1). EOC is chemosensitive, with a response rate to single agents varying from 10% to 45%. Alkylating agents, doxorubicin, hexamethylmelamine, cisplatin, and other agents have all been shown to have activity against the disease (2).

A number of studies have shown that patients who undergo debulking of their tumors to < 2 cm, together with bilateral salpingo-oophorectomy, total abdominal hysterectomy, and omentectomy, fare better than those in whom optimum debulking is not carried out (3). When this optimally debulked group of patients is subsequently treated with chemotherapy, median survival is > 24 months, with about 30%-40% of the patients experiencing a 3-year survival (4). Long-term follow-up of patients treated in this way would suggest that these patients may relapse, and it is doubtful that even at 7-10 years after treatment patients can be considered to be cured (5). In this setting, the subjective toxicity of treatment assumes importance. Modern therapeutic approaches are directed to achieving the maximum of remissions, with prolonged remission duration, while attempting to reduce side effects.

New chemotherapy agents with reduced toxicity but activity in EOC are therefore being sought. Mitoxantrone is an anthracenedione with activity against a wide range of tumors and few subjective side effects (6). It was therefore decided to carry out a study to determine the activity of mitoxantrone against EOC. The patients chosen were those who had failed initial therapy at this center, usually with cisplatin either alone or in combination, or those whose clinical condition was thought to be unsuitable for initial treatment with more intensive therapy.

PATIENTS AND METHODS

Forty-six patients were entered in the phase II study of single-agent mitoxantrone between August 1984 and October 1985. Patient characteristics are shown in table 1.

All patients had received previous treatment with the exception of five patients in whom intensive cisplatin-containing regimens were contraindicated because of impairment of renal function. The numbers of patients treated with the various first-line regimens are shown in table 1. Five patients had received previous treatment with doxorubicin and cisplatin in combination.

Patients were entered in the study if they had biopsy-

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[5] We thank all the clinicians who contributed patients to these studies, and Cyanamid International, which supported the study and supplied the drug.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	46
Age (yrs)	
< 30	1
30–50	8
> 50	37
Baseline performance status*	
1	2
2	42
3	2
Histology	
Serous	27
Mucinous	5
Endometrioid	3
Adenocarcinoma	11
Previous treatment	
Cisplatin alone	10
Cisplatin + alkylating agent	23
Cisplatin + doxorubicin	5
Alkylating agent only	3
None	5
Radiotherapy†	6
Evaluable disease	
Pelvic mass	23
Abdominal mass	15
Liver metastases	3
Lung metastases	1
Lymph nodes	3
Skin metastases	1

*WHO criteria.
†Given in addition to chemotherapy.

proven EOC or failure of first-line chemotherapy or were unsuitable for intensive first-line chemotherapy, no medical or cardiac contraindications to treatment, no history of other malignancy except nonmelanomatous skin cancer, and no anticancer treatment for at least 3 weeks (6 weeks for mitomycin, nitrosoureas, or radiotherapy). All patients had measurable and evaluable disease.

Mitoxantrone was administered at a dose of 14 mg/m^2 as an iv bolus over 90 seconds every 3 weeks. In the absence of significant myelotoxicity, the dose was increased by 2 mg/m^2 every second course. If myelotoxicity occurred (wbc count < 3000/mm^3; platelet count < 100,000/mm^3), then the dose was reduced by 2 mg/m^2. The doses administered and the number of patients treated at each dose are shown in table 2.

Responses were assessed using International Union Against Cancer criteria (7). Acute toxicity was assessed using World Health Organization (WHO) criteria (7). In addition, to evaluate any acute cardiotoxicity associated with the bolus injection, 13 patients had ECG recordings for 10 minutes prior to, during, and 10 minutes after each injection on a total of 31 occasions.

TABLE 2.—Cycles of mitoxantrone

Cycle dose (mg/m^2)	No. of cycles	No. of patients
10	3	1
12	15	4
14	79	46
16	38	14
18	1	1
20	10	1

RESULTS

Response

Forty-six patients were eligible and evaluable for response. Two patients achieved complete response and ten achieved partial response. Twelve patients had stable disease during the time that they were receiving treatment. Nineteen patients had disease progression and three received only one course of treatment, two because of rapidly progressive disease. The third patient died of gastrointestinal perforation. The patient had a large mass of metastatic ovarian cancer on the lesser curve of the stomach and was admitted as an emergency because of tumor resolution and perforation of this lesion following treatment. Despite emergency surgery, the patient died. Overall, the response rate was 26% (12 responses among 46 patients).

Responses were seen in patients who had been previously treated with cisplatin, doxorubicin, and alkylating agents (table 3). Eight of the responses occurred in patients treated at a dose ≥ 14 mg/m^2. Four of the responses occurred at a dose of 12 mg/m^2. No patient who failed to respond at a lower dose subsequently responded to a higher dose.

The median duration of response was 24 weeks (range, 10–30), and the median duration of stable disease was 15 weeks (range, 8–33).

Toxicity

The nonhematological toxicity of single-agent mitoxantrone is shown in table 4. This was assessed in 44 patients. In two patients it was not possible to assess toxicity because of early death due to progressive disease. Subjective toxicity was mild; only two patients experienced nausea and vomiting that required fluid replacement therapy and only one had alopecia which required a wig. There were ten cases of infection. These were either urinary tract infections or chest infections and there were no cases of septicemia. Examination of the ECGs taken prior to, during, and 10 minutes after the bolus injection showed no evidence of dysrhythmias or any other acute cardiological changes.

Myelosuppression was the dose-limiting toxic effect for this regimen. The median wbc and platelet counts fell progressively with treatment. The number of patients who required dose modification at each course is shown in table 5. There was an attempt to increase the dose if no toxicity was seen. It was possible to increase the dose in 16 patients, and six of these received two or more courses at the higher doses. One patient with stable disease received ten courses at 20 mg/m^2. A total

TABLE 3.—Response with respect to previous treatment

Response	Overall response	Previous treatment			
		None	Cisplatin	Cisplatin + other	Cisplatin + doxorubicin
Complete	2	0	2	0	0
Partial	10	2	1	6	1
Stable disease	12	1	3	5	3
Disease progression or early death	22	2	4	15	1

TABLE 4.—WHO nonhematological toxicity grades of single-agent mitoxantrone ($n = 44$)*

Toxicity	WHO grade				
	0	1	2	3	4
Nausea/vomiting	5	14	23	2	0
Alopecia	21	14	8	1	0
Diarrhea	25	7	11	1	0
Infection	34	2	7	1	0
Anxiety/malaise	22	16	5	0	1

*Values = No. of patients.

TABLE 5.—Dose modification due to myelosuppression*

Cycle No.	Treatment modification	
	No	Yes
1	46	0
2	28	5
3	21	4
4	13	7
5	9	6
6	3	6
7	3	1
8	1	1
9	2	0
10	0	1

*Values = No. of patients.

of 46 patients were studied to obtain an accurate response rate and to investigate the potential for dose escalation.

DISCUSSION

This study was carried out to establish whether mitoxantrone has activity against EOC. Two previous phase II studies of mitoxantrone suggested minimal activity (8,9). The patient populations in these studies were different from the present study. Twenty-one of 26 evaluable patients in the study by Muss et al (8) had received previous doxorubicin, as did 85% of the patients in the study by Hilgers et al (9). Sixteen of 33 patients in the latter study had received > 400 mg/m^2 of doxorubicin. Additionally, the patients had been heavily pretreated, and 80% of the patients in the study by Hilgers et al (9) were receiving the drug as third-line or greater therapy. The drug dose used in these studies varied from 10 to 12 mg/m^2. Although some responses were seen in the present study at 12 mg/m^2, there is evidence of a dose-response curve with mitoxantrone (6), and therefore the higher dose used in this study of 14 mg/m^2 might be expected to cause more responses. In this present study, patients had been exposed to as few drugs as possible prior to being entered in the study. Nonetheless, responses were seen in patients who had been exposed to cisplatin, doxorubicin, and alkylating agents, and an overall response rate of 26% in this setting indicates definite activity for the drug.

Toxicity was acceptable. There were few subjective side effects, and no patient refused treatment. ECGs were taken during the bolus injection to ensure that there was no evidence of acute cardiotoxicity such as dysrhythmias with this method of administration. The major toxic effect was myelosuppression. There was some evidence that this was cumulative, but the number of patients receiving more than six courses of drug was small. If the drug was to be used in combination, this could become the dose-limiting toxic effect.

The results of this study, showing undoubted evidence of activity for mitoxantrone, compare favorably with the results of other studies which have evaluated active single agents at relapse in EOC (2). The toxicity profile would suggest that it could be combined with other active drugs in the treatment of EOC, such as cisplatin. Further work is required to define the role of mitoxantrone as first-line therapy.

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Activity of Bisantrene in Refractory Lymphoma[1,2]

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A total of 50 evaluable adult patients with relapsing lymphoma and two with Hodgkin's disease received bisantrene by central iv catheter every 3 weeks, at doses of 350 mg/m² for patients with adequate marrow reserve and 300 mg/m² for those with compromised marrow reserve. The overall response rate was 30% for 50 patients with malignant lymphoma, including seven complete remissions (14%) and eight partial remissions (16%). Neither patient with Hodgkin's disease responded. The 16 patients with follicular lymphomas did particularly well, with 31% attaining complete remission and 25% attaining partial remission. Five of seven complete responders remain in remission at 16–24+ months; two have relapsed, at 13 and 20 months. Leukopenia, of brief duration, was the most common toxic effect. Fever during neutropenia occurred in 23% of courses. There was minimal cardiotoxicity with cumulative doses of up to 7080 mg/m² of bisantrene. Bisantrene is an effective and well-tolerated agent for the treatment of malignant lymphoma. [Cancer Treat Rep 71:631–633, 1987]

Bisantrene is an anthracenedicarboxaldehyde hydrazone that acts, at least in part, by DNA intercalation (1). It has shown promising activity in preclinical studies, including a number of animal tumor systems and the human tumor stem cell assay (2). In L5178Y murine lymphoma cells, bisantrene is four times more potent than doxorubicin in inhibiting incorporation of tritiated thymidine into DNA (3). In dogs, it has been shown to have less cardiac toxicity than doxorubicin (4).

In phase I trials, the dose-limiting toxicity was myelosuppression, and the optimum dose was 260–280 mg/m² every 3–4 weeks (5,6). Other toxic effects included phlebitis when administered by peripheral vein because of its low solubility at physiological pH (7), febrile reactions, and rare anaphylactoid reactions (8). Phase I trials suggested antitumor activity in lymphoma, melanoma, and carcinoma of the kidney, lung, and bladder (5,6). In phase II studies, little activity has been seen in extensively treated patients with melanoma or lung, head and neck, renal, or ovarian cancers. Responses have been reported in patients with breast cancer (9,10) and lymphoma (11).

This report summarizes our experience with 52 patients with lymphoma who received bisantrene.

MATERIALS AND METHODS

Between May 1983 and September 1984, 53 patients with malignant lymphoma and three with Hodgkin's disease were registered on study. All patients had measurable disease and were refractory to standard chemotherapy regimens. Patients were required to have bilirubin < 2.0 mg/dl, creatinine < 2.5 mg/dl, and adequate bone marrow function defined as a granulocyte count of > 2000 cells/mm³ and platelet count $> 100{,}000$/mm³; exceptions were made for patients with life-threatening problems secondary to their lymphoma. The histology was classified according to the International Working Formulation (12) by one of our staff hematopathologists.

Bisantrene was administered iv over 2 hours every 21 days by central catheter. The starting dose for good-risk patients (bilirubin < 2.0 mg/dl and prior good tolerance to chemotherapy) was 350 mg/m²; those with poor risk received 300 mg/m² as a starting dose. Subsequent doses were increased by 20% if the lowest granulocyte count was > 1000 cells/mm³ and the lowest platelet count was $> 75{,}000$/mm³; doses were decreased by 20% if the lowest granulocyte count was < 500 cells/mm³, the lowest platelet count was $< 50{,}000$/mm³, or if there was an episode of infection or hemorrhage.

Complete remission (CR) was defined as disappearance of disease for > 2 months with documentation by repeating all examinations that were abnormal prior to therapy. *Partial remission* (PR) was defined as a $> 50\%$ decrease of the sum of the products of the diameters of all measurable disease. *Minor response* was defined as an objective decrease of $< 50\%$, and *no response* was defined as no change or increase of disease.

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[4] We thank Linda Holmes, RN, for her help with data management and Linda Hill for her assistance in preparation of this manuscript.
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RESULTS

Of 56 patients registered on study, four were not evaluable: one returned home without receiving the drug; one refused repeat bone marrow examination after surviving a complicated episode of tumor lysis; one with pretreatment neutropenia and circulating lymphoma cells refused reevaluation because the first course was complicated by an infection; and one with Hodgkin's disease refused reevaluation after one course because the appointment was inconvenient.

The 52 evaluable patients received a median of two courses of bisantrene (range, one to 19 courses). The median age of the patients was 50 years (range, 19–71 years). Twenty-eight were men. Prior therapy consisted of a median of three regimens (range, one to six) and a median of nine agents (range, three to 15). All but two patients had received prior doxorubicin; 17 were clearly refractory to doxorubicin. Clinical features of the 52 evaluable patients are summarized in table 1.

Fifteen of 50 patients (30%) with malignant lymphoma achieved a major response, seven a CR and eight a PR, as illustrated by histologic type in table 2. Neither patient with Hodgkin's disease responded. The subset of patients with follicular lymphomas did particularly well, with 31% CRs and 25% PRs. The duration of CR ranged from 13 to 24+ months (table 2). The median duration of PR was 4.5 months (range, 2–13+). The activity of bisantrene according to prior therapy is noted in table 2, and illustrates that those with extensive prior therapy and those not previously achieving CR had a lower response rate than patients with a better prior treatment history. Notably, two patients whose maximum response to prior therapy was PR did achieve CR on bisantrene. Better responses to bisantrene than to prior doxorubicin-based combinations were noted in two patients, and four patients who progressed on doxorubicin-based combinations achieved minor responses following bisantrene.

TABLE 1.—Clinical features of 52 evaluable patients

Feature	No. of patients	%
Histology		
Low grade	16	31
Intermediate grade	31	60
High grade	3	6
Hodgkin's	2	4
Performance status (Zubrod scale)		
0	8	15
1	31	60
2	11	21
3	2	4
Sites of disease		
Nodal only	17	33
Extranodal		
Marrow only	10	19
Other extranodal site	13	25
≥ 2 extranodal sites	12	23
Prior therapy		
Combination chemotherapy	52	100
Doxorubicin combinations	50	96
Radiotherapy	28	54

TABLE 2.—Response according to histology and prior treatment history

	No. of patients	Response*		
		CR	PR	MR
By cell type†				
Small lymphocytic	4	—	—	—
FSC and FM	12	3 (16+,17+,19+)	4	1
FLC	4	2 (20,24+)	—	—
DSC and DM	4	1 (13)	—	—
DLC	23	1 (22+)	4	2
Small noncleaved	3	—	—	—
Hodgkin's	2	—	—	—
By response to initial therapy				
CR	27	5	5	2
PR	19	2	2	1
Failure	6	—	1	—
By No. of prior relapses				
1	5	1	2	—
2	12	4	1	—
3	14	2	1	1
≥ 4	21	—	4	2

*CR duration in mos is indicated in parentheses. MR = minor response.

†F = follicular; SC = small cleaved; M = mixed; LC = large cell; and D = diffuse.

Myelosuppression was the most frequent toxic effect. Excluding 12 patients with preexisting cytopenias or extensive marrow involvement by lymphoma, there were 20 patients who received an initial bisantrene dose of 300 mg/m^2, and 20 who received an initial dose of 350 mg/m^2. Neutropenia (granulocyte count < 1000 cells/mm^3) was documented in 85% of patients at the lower dose level, and 95% of patients at the higher dose level. Thrombocytopenia (platelet count < 100,000 cells/mm^3) was documented in 20% of patients at the lower dose level, and 40% at the higher dose level. The median time to hematologic recovery was 21 days (range, 17–28 days). There was no evidence of cumulative marrow toxicity. Of the 12 patients with pretreatment marrow dysfunction, 67% had thrombocytopenia and 100% had neutropenia during therapy; six had febrile episodes and four of these were documented infections. The remaining patients had febrile episodes while neutropenic in 23% of courses, one-half of which were documented infections and one of which was attributed to a subclavian catheter. Other frequent toxic effects included drug-related fever on Days 1–2, mild nausea and vomiting, and malaise. Phlebitis occurred in the one patient who received bisantrene by peripheral vein but in none of the other patients. Tumor lysis occurred in one patient. No alopecia or anaphylactoid reactions occurred.

The potential for cardiotoxicity with multiple doses of bisantrene appeared to be minimal. Table 3 illustrates data for the 12 patients who received more than five courses of bisantrene. Two had a drop of cardiac ejec-

TABLE 3.—Drug doses and cardiac monitoring of 12 patients who received > 5 courses of bisantrene

Prior doxorubicin (mg/m^2)	Total bisantrene (mg/m^2)	Cardiac ejection fraction (%) by gated radionuclide scan	
		Before bisantrene	After bisantrene
120	2146	71	69
180	2250	—	74
460	2250	65	61
400	3600	64	48
416	6650	76	65
400	2550	68	59
250	4690	60	47
438	3360	68	67
300	4550	—	63
369	5100	75	69
407	5696	78	71
0	7080	64	76

tion fraction of > 15% or to < 55%; one of these developed congestive heart failure which responded to medical management. One other patient who received four courses of bisantrene after a total doxorubicin dose of 390 mg/m^2 also had a 15% drop of ejection fraction (from 76% to 61%).

DISCUSSION

Bisantrene was found to be active in refractory malignant lymphoma: one-third of the patients responded. Moreover, there have been several durable CRs, including two with intermediate-grade histologies and a consequent low likelihood of durable remissions following salvage therapy (13). Partial non-cross-resistance with doxorubicin was suggested by better responses to bisantrene than to prior doxorubicin-based combinations in several patients, although these patients had not received fully comparable high-dose doxorubicin used as a single agent. The inferior results reported by Miller et al (11,14) in lymphoma patients may be due in part to the lower doses employed in those studies (208–260 mg/m^2). In addition, our trial included more patients with low-grade lymphomas who appeared to be more sensitive to bisantrene than other patients, as Miller et al (11) speculated.

The major toxic effect in this trial was myelosuppression, which was of consistently brief duration. Twenty-three percent of courses were complicated by fever with neutropenia. Phlebitis was not a problem in patients who received bisantrene by central venous catheter. The excellent tolerance of cumulative doses of up to 7080 mg/m^2 of bisantrene (table 3) suggests that bisantrene has less cardiotoxicity than doxorubicin. The activity of bisantrene in lymphoma patients justifies consideration of further trials with this agent.

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BRIEF REPORTS

Continuous Sc Infusion of Narcotics Using a Portable Disposable Device in Patients With Advanced Cancer[1]

Eduardo Bruera,[2,3,*] Carleen Brenneis,[2] Mary Michaud,[2] Sharon Chadwick,[2] and R. N. MacDonald[2,4]

Fifty-six patients with chronic pain due to advanced cancer were treated with an sc infusion of morphone (34 patients) or hydromorphone (22 patients) using a portable disposable infusor. The infusion was maintained for 26 ± 14 days. Twenty-five patients (45%) were discharged to the home for a mean period of 18 days (range, 2–138). The mean daily increase in dose was 2.6% ± 1.4% of the initial dose. The sc site needed to be changed every 8 days (range, 2–31). Local toxicity consisted of infection in two patients (3%), chemical irritation in three (5%), and bleeding in one (2%). After 48 hours of treatment, 54 of 56 patients (96%) preferred the sc infusion to their previous parenteral treatment. We conclude that this is a safe and simple method for the administration of narcotics to inpatients and outpatients. [Cancer Treat Rep 71:635–637, 1987]

Many patients with advanced cancer need parenteral narcotics for adequate pain control (1,2). The iv route, either as intermittent injections or continuous infusion, is expensive and uncomfortable for the patient and may prevent the patient from returning home. A recent case report described successful pain control with an sc infusion of morphine (3). We report the treatment of 56 patients with cancer pain with a continuous sc infusion of morphine or hydromorphone using a portable disposable device.

PATIENTS AND METHODS

Fifty-six consecutive patients who had pain due to cancer and were receiving parenteral narcotics as intermittent injections (14 patients) or continuous iv infusion (42 patients) were treated. The only criteria for admission were the need for parenteral narcotics and the possibility of achieving good pain control iv before starting the sc infusion. All patients started a continuous sc infusion of narcotics using the Travenol infusor. This is a plastic disposable cylinder that measures 16 cm in length and 3 cm in diameter, weighs 90 g when full, and infuses a total volume of 48 ml/day at a fixed rate of 2 ml/hour (fig 1). The infusion was administered underneath the skin of the subclavicular region using a #25 Butterfly needle. In all patients adequate pain control was achieved with iv narcotics before starting the sc infusion. All of the patients continued sc with the same analgesic they were receiving iv (either morphine or hydromorphone) at the same initial daily dose. All patients were admitted to the hospital at the time of initiation of the sc infusion. They were followed up daily by one of the investigators while in the hospital and twice a week when they were discharged. Table 1 summarizes patient characteristics and treatment results. Parenteral narcotics have been suggested to promote the development of tolerance faster than when given orally (4–6). For this reason we measured the daily increase in the dose of narcotics in 38 patients who received the infusion for > 4 days and underwent no other procedures for pain control. The change in the daily dose of narcotics was expressed as a percentage of the initial sc dose,

$$\% \text{ daily increase} = \frac{\text{final dose} - \text{initial dose}}{\text{initial dose}} \times 100.$$

RESULTS

The mean duration of infusion was 26 ± 14 days. Twenty-six of 56 patients (45%) were discharged to the

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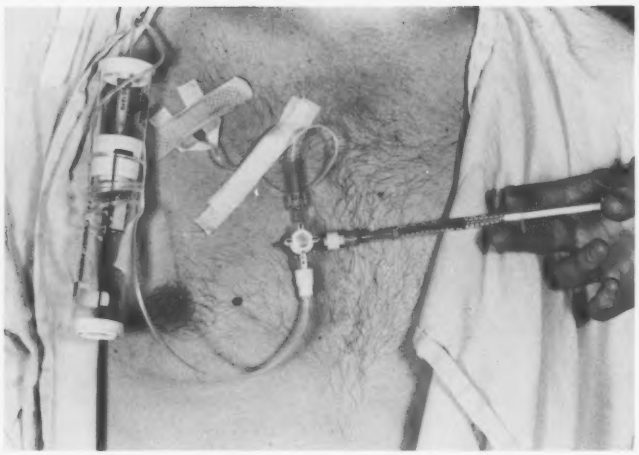

FIGURE 1.—Travenol infusor attached to patient's clothes, infusing sc to the subclavicular region. 3-way stopcock allows administration of extra doses of analgesics or other medications.

home on an sc infusion for a median period of 18 days (range, 2–138). The sc infusion was discontinued in 12 patients because they needed an iv line for other reasons (hydration, administration of drugs, or discharge to another hospital) and in eight patients because they could restart oral narcotics (usually after radiotherapy, chemotherapy, or neurosurgical procedures); 36 patients died on the sc infusion.

After 48 hours of sc infusion, 54 of 56 patients (96%) preferred the sc infusion to their previous iv treatment (the remaining two patients expressed no preference). The main reason for the choice was increased comfort in all 54 patients. Additional reasons for the choice were easier mobility (32 patients) and better pain control (18 patients).

Because this device had not been used sc before, we carefully measured the rate of infusion in 297 consecutive pumps; it was found to be 2.2 ± 0.2 ml/hour (range, 2–2.4), suggesting that there is a trend to overpump by approximately 10%. This difference in rate was not considered relevant for narcotic use.

In the last 40 consecutive patients, the site of infusion was checked daily while the patient was admitted to the hospital and was not changed unless the patient experienced pain, itchiness, or swelling in the area. The median duration of the sc site was found to be eight days (range, 2–31). The first 16 patients had their infusion site changed regularly every 3 days.

Eight patients (14%) developed severe drowsiness (three patients) or confusion (five) that improved on adjustment of the dose of infusion. One patient developed respiratory depression during the sc infusion that improved with discontinuation of hydromorphone for 2 hours and reduction in the dose from 25 to 12 mg/day (an 85-year-old patient with advanced breast cancer who had episodes of confusion before starting the sc infusion; renal and liver functions and electrolytes were normal). It was not necessary to discontinue the sc infusion because of toxicity in any patient.

Local toxicity to the sc infusion consisted of infection in two patients (3%, both patients with severe neutropenia), chemical irritation in three patients (5%), and bleeding in one patient (2%).

A three-way stopcock permitted the administration of extra doses of narcotics using the same site of infusion (fig 1). The extra dose consisted of the equivalent of 2 hours of infusion and was received by our patients a mean of 1.2 ± 1 times/day.

The main daily increase was 2.6% ± 1.4%. Only six patients (16%) had a daily increase in their dose of > 5%. These six patients did not differ from the rest in age,

TABLE 1.—Patient characteristics and treatment results*

Characteristic	No. of patients
Total	56
Sex (male/female)	24/32
Age (yrs): 54 ± 7	
Primary tumor	
Breast cancer	22
Lung cancer	6
Colon cancer	3
Prostate cancer	5
Kidney cancer	5
Sarcoma	4
Other	11

*Mean duration of infusion (days), 29 ± 15; No. of patients discharged home, 25 (45%); mean maximal dose of morphine (34 patients), 235 ± 105 mg/day; and mean maximal dose of hydromorphone (22 patients), 126 ± 60 mg/day.

sex, stage of disease, or initial daily dose of narcotics. Adequate pain control was maintained in the patients by frequently increasing the dose of narcotics.

DISCUSSION

Our data suggest that morphine and hydromorphone can be safely administered sc. The Travenol infusor is a simple device that can be safely used in inpatients or outpatients. Although the site of infusion needed to be changed approximately once a week, special precautions (ie, changing site every 3 days) should be taken in patients with neutropenia. No difference was found between morphine and hydromorphone in pain control or toxicity. However, hydromorphone, being more soluble, allows the administration of a higher daily dose in the limited volume (48 ml/day) of the infusor. The reason why some patients required frequent dose increases cannot be determined. It may be due to the rapid development of tolerance or to progressive disease. These patients are poor candidates for home discharge because of the frequent dose changes that are needed to maintain adequate pain control.

The Travenol infusor has some advantages when compared to battery-operated pumps: it is very light (90 g when full), very simple (requires minimal teaching and patients and nurses feel very comfortable with it), and immediately available when needed (no need to wait for a battery-operated pump to be available; no need for specially trained nurses). Its main disadvantages are its price ($25 Canadian per day) and the need to change it on a daily basis.

A comparison between the infusor and other nondisposable devices, in terms of effectiveness and cost, is under way. This method of administration of narcotics can significantly improve the quality of life of patients by spacing the need for iv lines (free limbs, easier ambulation) and by allowing some patients to return home.

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BRIEF REPORTS

Salvage Chemotherapy for Non-Hodgkin's Lymphoma With a Combination of Mitoxantrone, Etoposide, Cisplatin, and Prednisolone[1]

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We tested the combination of mitoxantrone, etoposide, cisplatin, and prednisolone as salvage treatment for patients with advanced aggressive non-Hodgkin's lymphoma. Among 18 evaluable patients, four complete and seven partial responses were observed, for an overall response rate of 61%. The median duration of response was 36 weeks (range, 11–58). The median survival time from the beginning of the regimen was 52 weeks for responders and 24 weeks for nonresponders. Myelosuppression was the major dose-limiting toxic effect; however, it was generally well-tolerated. These results indicate that the combination regimen can play a role in the treatment of relapsed or resistant non-Hodgkin's lymphoma. [Cancer Treat Rep 71:639–641, 1987]

In patients with aggressive non-Hodgkin's lymphoma (NHL) receiving optimal front-line chemotherapy, approximately 30% of those will fail to achieve complete response (CR) and a substantial proportion of complete responders will eventually relapse. An effective salvage regimen therefore is still in great demand. Reports of phase II trials of mitoxantrone, etoposide, and cisplatin indicate that these drugs individually retain substantial activity against NHL resistant to conventional drugs (1–3). Etoposide and cisplatin in combination have been shown to have synergism in the mouse P388 leukemia model (4) and to give an additive effect in a human lymphoma cell line (5). Furthermore, we have demonstrated a lack of cross-resistance between doxorubicin and mitoxantrone using a doxorubicin-resistant human small cell lung cancer cell line (6). We therefore designed this study to assess the efficacy of a four-drug combination of mitoxantrone, etoposide, cisplatin, and prednisolone as a salvage regimen for resistant or relapsed NHL.

MATERIALS AND METHODS

Between March 1984 and April 1986, 19 patients with advanced aggressive NHL were evaluated for this study after their disease failed to respond to or relapsed after front-line chemotherapy. Eligibility criteria included intermediate or high-grade NHL of the Working Classification (7); no prior exposure to any of the study drugs except prednisolone; presence of measurable disease; ambulatory status; and adequate renal, hepatic, and bone marrow functions unless involved by tumor.

The treatment program was as follows: mitoxantrone at a dose of 8 mg/m^2 iv on Day 1; etoposide at 140 mg/m^2 orally on Days 1, 3, and 5; cisplatin at 20 mg/m^2 iv infused over 30 minutes on Days 1–5; and prednisolone at 40 mg/m^2 orally on Days 1–5. All patients were hospitalized for therapy. Patients were hydrated with 2 L of normal saline/day throughout the period of therapy. Mannitol was not given. Courses were to be repeated every 4 weeks and continued until disease progression occurred or toxicity became intolerable. Doses of each drug except prednisolone were attenuated by 25% for a wbc count of 3000–3500/μl and/or a platelet count of 75,000–100,000/μl on the day of therapy and when a patient encountered infection-related fever in the previous course. For a wbc count $< 3000/\mu$l and/or a platelet count $< 75{,}000/\mu$l, all drugs were withheld for 1 week. However, if a patient had low counts secondary to bone marrow involvement and responded well, the patient received a planned dose of all the drugs in the first two courses. Cisplatin was withheld if creatinine clearance fell to $<$ 40 ml/minute.

Patients were evaluated for tumor response after completion of each course by physical examination, fol-

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low-up x-ray or lymphangiogram, computerized tomographic scans of the abdomen, and bone marrow biopsy and/or other studies, as indicated. CR was defined as the disappearance of all clinical evidence of tumor for at least 4 weeks. Partial response (PR) was defined as a > 50% reduction in the product of two perpendicular diameters of all measurable lesions for at least 4 weeks.

RESULTS

Nineteen patients received the combination as described. One patient died of massive gastrointestinal bleeding on the fifth day of the first course of therapy and was not evaluable for tumor response and toxicity. Pretreatment patient characteristics and response are shown in table 1. All patients had received CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or CHOP-Bleo (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin) previously. At the time of entry six patients had nodal disease alone and 12 had extranodal disease, including nine with bone marrow involvement. A total of 72 courses of therapy were administered in the 18 patients, with a median number of four courses per patient (range, one to ten courses).

Of the 18 evaluable patients, four CRs and seven PRs were observed, for an overall response rate of 61%. CR was documented in three of six patients whose disease was confined to lymph nodes alone, but in only one of 12 patients with extranodal disease. As for response to prior chemotherapy, there were four CRs and two PRs among nine patients who had relapsed after discontinuation of therapy, while there were five PRs alone among nine patients with refractory disease, including four partial responders who had developed progressive disease while receiving therapy. The durations of response were 36, 40+, 41, and 55 weeks for CR and 11, 16, 24, 28, 34, 40+, and 58 weeks for PR. The median survival time from the beginning of the regimen was 52 weeks for responders and 24 weeks for nonresponders.

The major toxic effect was myelosuppression, which was severe for those with massive marrow involvement. All patients received a planned first dose, but only 28 (52%) of 54 repeated courses could be administered at a full dose. Wbc count nadirs < 500/μl or platelet count nadirs < 50,000/μl were seen in eight patients. One patient who had massive marrow involvement died of septicemia while neutropenic after the second course. A decreased creatinine clearance (< 40 ml/minute) was documented in two patients; however, it was reversible for both. Nausea and vomiting were common, but generally well-tolerated.

TABLE 1.—Patient characteristics and response*

Characteristic	No. of patients	Response		
		CR	PR	None
Evaluated	18	4	7	7
Median age in yrs (range): 56 (32-71)		45	61	49
Sex				
Male	12	4	4	4
Female	6	0	3	3
Histology†				
Diffuse, small cleaved	4	0	2	2
Diffuse, mixed	7	3	2	2
Diffuse, large	4	1	1	2
Large cell, immunoblastic	3	0	2	1
Sites of disease				
Systemic, nodal	6	3	2	1
Nodal and extranodal	12 (9)	1 (1)	5 (3)	6 (5)
Response to prior chemotherapy				
Relapsed	9	4	2	3
Refractory	9	0	5	4

*Unless otherwise specified, values = No. of patients. Nos. in parentheses = No. of patients with bone marrow involvement.
†Histology according to the Working Classification.

DISCUSSION

With a combination of etoposide and cisplatin, Judson and Wiltshaw (8) reported an overall response rate of 53% in 17 patients with previously treated NHL, while a report by Cavalli (9) was less impressive, giving a response rate of 29% in the same group of patients. The duration of response was reported to be 12-48 weeks in the former and a median of 12 weeks in the latter. The observed response rate and the duration of response in our series are more favorable compared with those of the etoposide and cisplatin combination, suggesting an enhanced therapeutic effect of mitoxantrone in this program. Substantial myelotoxicity was not unexpected in this group of patients who had been heavily pretreated, the majority of whom were affected with marrow involvement. Although one patient died of septicemia, the toxicity was within a tolerable limit for the rest. These results indicate that the combination regimen can play a role in the treatment of relapsed or resistant NHL and warrant further clinical trials.

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BRIEF REPORTS

Lack of Benefit of Adjunctive Chemotherapy in Stage I Malignant Melanoma: A Southwest Oncology Group Study[1,2]

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In 1975, the Southwest Oncology Group initiated an adjuvant study in localized malignant melanoma testing the value of aggressive chemotherapy using carmustine, hydroxyurea, and dacarbazine versus a control arm. Median disease-free survival was 7.1 years for the control arm and 6 years for the treatment arm. Survival was identical for both arms with 65% of the patients alive at 6 years. We conclude that carmustine, hydroxyurea, and dacarbazine chemotherapy is of no value as adjuvant treatment for localized melanoma. [Cancer Treat Rep 71:643-644, 1987]

In 1975, the Southwest Oncology Group initiated a study to test the value of using aggressive adjunctive chemotherapy in localized melanoma with a poor prognosis. The combination of carmustine (BCNU), hydroxyurea, and dacarbazine (BHD) was chosen as the chemotherapy because of its effectiveness (1) in the treatment of advanced melanoma. Full doses of these three drugs were given: BCNU at 150 mg/m^2 iv every 8 weeks; hydroxyurea at 1500 mg/m^2 orally on Days 1-5 every 4 weeks; and dacarbazine at 150 mg/m^2 iv on Days 1-5 every 4 weeks. The chemotherapy was given monthly for 12 courses.

One hundred twenty-three patients with localized melanoma were randomized to receive either the 3-drug combination BHD (60 patients) or to observation only (63 patients). One patient in each group was declared ineligible. The two groups of patients were equally matched as to prognostic variables (table 1). All patients had lesions that penetrated to the papillary dermis or deeper or had mucous membrane presentation.

Patients were all initially treated with wide local excision. Lymph node dissection was not routinely carried out.

Toxic effects consisted of nausea, vomiting, and hematological toxicity for those treated in the BHD arm. Thrombocytopenia $< 75{,}000$ cells/mm^3 occurred in 28% of patients, and leukopenia < 3000 cells/mm^3 also occurred in 28% of those treated.

The median disease-free interval was 6 years for the BHD arm and 7.1 years for the control arm. The median survival time for the two arms has not yet been reached, but the rate is identical, 65% at 6 years.

Results of this study are disappointing in that the disease-free interval and survival are the same for the treated and untreated arms.

There are other studies (2-4) using dacarbazine alone and at higher doses that also failed to show any advantage to adjunctive chemotherapy.

The benefits of the BHD regimen in advanced melanoma are reported as a response rate of 28% and an average survival of 6 months (1). In breast cancer, a disease where adjunctive chemotherapy has shown bene-

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TABLE 1.—Distribution of patient characteristics by treatment arm

	No. of patients (%)		
	BHD	Control	Total
Age (yrs)			
< 40	16 (27)	16 (26)	32 (26)
40–59	26 (44)	33 (53)	59 (49)
60–79	15 (25)	10 (16)	25 (21)
≥ 80	2 (3)	3 (5)	5 (4)
Sex			
Male	30 (53)	38 (64)	68 (59)
Female	27 (47)	21 (36)	48 (41)
Depth			
≤ Papillary dermis	8 (14)	5 (8)	13 (11)
Reticulum dermis	30 (51)	32 (52)	62 (51)
Subcutaneous fat	9 (15)	10 (16)	19 (16)
Unknown	12 (20)	15 (24)	27 (22)
Type			
Superficial spread	21 (36)	16 (26)	37 (31)
Nodular	27 (46)	30 (48)	57 (47)
Unknown	11 (19)	16 (26)	27 (22)
Site			
Head and neck	11 (19)	5 (8)	16 (13)
Trunk	21 (36)	27 (44)	48 (40)
Extremity	17 (29)	21 (34)	38 (31)
Other*	5 (8)	5 (8)	10 (8)
Multiple	2 (3)	0 (0)	2 (2)
Unknown	3 (5)	4 (6)	7 (6)

*Includes eye and mucous cavities.

fits, the response rate in advanced disease is 50%–70%, with a mean survival of 22 months (5). One would suppose that a certain efficacy in advanced disease would be demonstrable before its value could be seen in the adjunctive setting.

A recent study (6) using the combination of vinblastine, bleomycin and cisplatin in advanced melanoma showed a response rate of 19% (complete + partial) and a short duration of response. This also casts doubt on the possible benefits of this new regimen in the adjuvant setting.

This study was not large enough (123 patients in two arms) to exclude the possibility that the chemotherapy regimen produces a clinically significant improvement in disease-free interval. At the same time, the data provide no indication that such a benefit exists.

Based on the above data, we conclude that there is no advantage in the use of the BHD combination as adjuvant therapy in stage I malignant melanoma. In view of the failure of the BHD regimen to reduce recurrence, the organized groups should continue to investigate methods of adjunctive treatment in malignant melanoma.

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BRIEF REPORTS

Total-Body Irradiation and High-Dose Teniposide: A Pilot Study in Bone Marrow Transplantation for Patients With Relapsed Acute Lymphoblastic Leukemia[1,2]

R. A. Krance,* S. J. Forman, and K. G. Blume[3]

Inadequate leukemic ablative therapy ending in leukemic relapse is the source of most failures following bone marrow transplantation. Attempting to improve pretransplant leukemic ablative therapy, we report on a pilot regimen that includes teniposide, the principal chemotherapy, and total-body irradiation. Ten patients with acute lymphoblastic leukemia in relapse underwent allogeneic bone marrow transplantation. All patients engrafted. Toxicity, primarily mucositis, was manageable. Two patients have survived for 1547 and 1988 days in continued remission. Eight patients have died: three from sepsis in the immediate posttransplant period, four with recurrent leukemia, and one with chronic graft-versus-host disease and pneumonia. We believe that these results support further trials with teniposide in pretransplant leukemic ablative therapies. [Cancer Treat Rep 71:645-647, 1987]

Bone marrow ablation followed by bone marrow transplantation (BMT) can produce prolonged continuous remission in 10%-60% of patients with acute lymphoblastic leukemia (ALL). This wide range for remission reflects, in part, the patient population under investigation, since patients transplanted in second remission fare better than patients transplanted in later remission or relapse (1). Several factors mitigate against the successful outcome of BMT, including infection, interstitial pneumonia, and graft-versus-host disease (GVHD); however, the most common reason for failure after BMT is leukemic relapse (1). Recurrence of leukemia almost invariably arises from host cells and usually retains the same cytogenetic abnormality that was present prior to BMT (2). Hence, relapse is ascribed to inadequate pretransplant leukemic ablative therapy. Therefore, to improve disease-free survival following BMT, better leukemic ablative treatment is needed.

The epipodophyllotoxin derivative teniposide (VM-26) is active against ALL in previously treated patients (3). Dose-limiting toxicity of VM-26 is due to bone marrow suppression. Since no other dose-limiting organ system toxicity has been demonstrated for VM-26, the potential of this agent in a leukemic ablative radiochemotherapy regimen is worth exploring. This report presents our experience using VM-26 in high-dose infusion (10 or 15 mg/kg) as part of a BMT preparatory regimen for ALL in relapse.

PATIENTS AND METHODS

Patients with ALL in relapse with an HLA-identical, MLC-compatible sibling donor were eligible for this study. Between March 1981 and June 1984 ten patients were entered. Their characteristics are summarized in table 1. Eight patients were treated with VM-26 (10 mg/kg) infused over 2 hours in 5% dextrose and water in one-half normal saline (at a final drug concentration of 1 mg/ml) on Day −5, relative to the day of BMT on Day 0. In addition, these patients received cytarabine (Ara-C) (5 mg/kg iv) on Days −8 and −3 and vincristine (Vcr) (0.02 mg/kg iv) on Day −1. Total-body irradiation (TBI) (1000 cGy) was administered in a single dose on Day −1. Two patients received VM-26 (15 mg/kg), infused as in the former regimen on Day −3, and fractionated TBI (total dose, 1320 cGy), delivered in 11 fractions on Day −7 to Day −4. The protocols for radiotherapy have been published elsewhere (1,4). GVHD prophylaxis and therapy were the same for all patients, consisting of methotrexate and prednisone (4). GVHD was graded according to accepted criteria (4). All patients were placed in reverse isolation and received antibiotics, hy-

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TABLE 1.—Patient characteristics prior to and outcome following BMT

UPN	Sex	Age at BMT (yrs)	ALL phenotype	Bone marrow status at transplant	Blasts in bone marrow (%)	Acute GVHD	Status
				*Schedule 1**			
104	M	28	T-cell	Induction failure	60	Inevaluable	Died on Day +18 with sepsis, severe mucositis, and caliceal hemorrhage
119	F	6	Non-T, non-B cell	2nd relapse	25	I	Alive in continuous remission on Day +1988
134	M	5	Not typed	2nd relapse	48	Inevaluable	Died on Day +18 with sepsis and severe mucositis
140	M	14	Non-T, non-B cell, TdT−	2nd relapse	63	II	Relapsed on Day +292; died on Day +382
159	F	28	Non-T, non-B cell, TdT+	1st relapse	45	Inevaluable	Died on Day +14 with sepsis and severe mucositis
163	M	7	Not typed	2nd relapse	12	0	Relapsed and died on Day +896
167	F	5	Non-T, non-B cell, Calla+	2nd relapse	10	0	Alive in continuous remission on Day +1547
173	F	36	Non-T, non-B cell, Calla+, TdT+	1st relapse	95	0	Relapsed on Day +230; died on Day +371
				Schedule 2†			
193	M	31	Non-T, non-B cell, Calla−	2nd relapse	23	III	Died on Day +417 with GVHD and pneumonia
247	F	11	Non-T, non-B cell, Calla−, TdT+	1st relapse	40	I	Relapsed on Day +102; died on Day +109

*Patients received VM-26 (10 mg/kg) on Day −5, relative to the day of BMT on Day 0; Ara-C (5 mg/kg) on Days −8 and −3; and Vcr (0.02 mg/kg) on Day −1; TBI (1000 cGy) was administered as a single dose on Day −1.

†Patients received VM-26 (15 mg/kg) on Day −3; TBI (1320 cGy) was administered in 11 fractions on Day −7 to Day −4.

peralimentation, and blood products (4), except that prophylactic granulocyte transfusions were not employed. The last two patients received nonabsorbable antibiotics for gastrointestinal tract sterilization (5).

RESULTS

As seen in table 1, two patients [Unique Patient Nos. (UPN) 119 and 167] are alive in continuous remission 4 and 5 years after BMT (date of evaluation, January 1, 1987). Neither patient has chronic GVHD; their Karnofsky score is 100. Three patients (UPNs 104, 134, and 159) died from sepsis in the immediate posttransplant period, two patients with *Escherichia coli* sepsis and one with *Klebsiella pneumoniae* and *Candida albicans* sepsis. One patient (UPN 193) died from pneumonia and chronic GVHD on Day 417 posttransplant. Four patients (UPNs 140, 163, 173, and 247) relapsed with leukemia on Days 102, 230, 292, and 896, respectively, after BMT. None of these four patients survived.

Bone marrow engraftment was documented for all patients by Day 15 posttransplant. Excluding the three patients who died from sepsis, the total wbc count was $\geq 1000/mm^3$ between 20 and 26 days posttransplant (mean, 22 days). All patients were febrile. Only the three patients who died from sepsis had specific pathogens identified. For the seven patients who survived > 3 weeks, the range of febrile days was 2–33 days (mean, 11). Therapeutic granulocyte transfusions were administered for clinical indication to six patients, including the three who died from sepsis. All patients developed mucositis, and in five patients the mucositis was severe (UPNs 104, 134, 159, 167, and 247).

Because three patients died from sepsis associated with severe mucositis, the BMT preparatory regimen was modified. Vcr and Ara-C were deleted and VM-26 was administered in an increased dose. TBI was delivered in a fractionated schedule instead of a single dose. Two patients were treated with this regimen and one developed severe mucositis. Because of the small numbers of patients, comparison between regimens was not possible.

CONCLUSION

Attempting to develop more effective leukemic ablative treatment and thus improve disease-free survival following BMT, we have piloted a new BMT preparatory regimen in which VM-26 is the primary chemotherapeutic agent. Two of ten patients with ALL in relapse treated with this new regimen survive. Although three patients died in the immediate posttransplant period with mucositis and sepsis, we do not judge this therapy as excessively toxic. Patients who undergo BMT

while in advanced relapse are more likely to experience early morbidity (4). Further, other possible indicators of an unduly toxic pretransplant regimen were absent. For the seven patients surviving > 2 weeks, the wbc count was $\geq 1000/mm^3$ by 22 days (mean). This recovery period is similar to that required for patients treated with more standard regimens (4). Finally, it was our impression that patients in this pilot study did not develop excessive GVHD or frequent pulmonary complications.

Barrett et al (6) have recently reported using VM-26 and TBI along with several other agents as a leukemic ablative regimen followed by allogeneic BMT. The actuarial relapse probability for 17 patients with ALL in second or subsequent remission is 43%. Because their regimen differs substantively from the one we report, comparison is unwarranted; nonetheless, their results taken with ours support further trials with VM-26 in pretransplant leukemic ablative regimens.

Concurrent with this pilot study, a collateral study was inaugurated for patients with acute nonlymphoblastic leukemia in relapse. Pretransplant therapy was identical except that etoposide was substituted for VM-26. We assessed the need for two leukemic ablative regimens on presumed antileukemic specificity for each agent. Subsequent reports indicate that neither epipodophyllotoxin has superior activity against acute leukemia of either phenotype (7,8). To increase the experience with a single therapy, we discontinued the VM-26 study and treated all patients undergoing BMT after first relapse with the etoposide regimen. Thirty-three patients with advanced acute leukemia have received this regimen followed by BMT and the actuarial disease-free survival rate is 43% (5).

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BRIEF REPORTS

Phase II Trial of Aminothiadiazole in Previously Treated and Untreated Patients With Advanced Colorectal Carcinoma: An Illinois Cancer Council Trial[1,2]

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Eighty-three patients with advanced colorectal carcinoma were entered on a phase II trial of weekly iv aminothiadiazole (125 mg/m^2) plus daily oral allopurinol (300 mg). There were five partial responses. Median survival of all patients on study was 36 weeks from entry (48 weeks for those without prior therapy and 34 weeks for those with previous chemotherapy). Toxicity was generally mild and consisted predominantly of stomatitis. In the dose given, aminothiadiazole has limited activity against metastatic colorectal cancer. [Cancer Treat Rep 71:649-650, 1987]

Aminothiadiazole (ATDA), an analog of the thiadiazole class of compounds structurally related to niacin, has been shown to have significant antitumor activity against murine leukemia and lung cancer models (1). The Illinois Cancer Council has completed a phase II trial of ATDA in patients with advanced colorectal carcinoma to evaluate its single-agent activity in that disease.

METHODS

Patients were eligible if they had histologically proven and measurable metastatic or recurrent colorectal carcinoma. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-3 and had normal wbc count, platelet count, serum BUN, creatinine, and bilirubin. ATDA was administered at a dose of 125 mg/m^2 iv weekly, along with daily oral allopurinol (300 mg) to prevent hyperuricemia, a known side effect of the drug (1). Treatment continued until the patient developed grade 2 stomatitis (2), at which time the ATDA was held for 1 week, then resumed at the same dose. Grade 3 stomatitis resulted in a 66% decrease in the subsequent dose. Toxicity and objective response criteria were as defined and published by the ECOG (2).

RESULTS

Pretreatment characteristics of the 83 patients entered on the trial are summarized in table 1. A large number of patients were enrolled to permit separate analyses of the previously treated with chemotherapy and the previously untreated subgroups and to evaluate impact on survival (3). Eighty-two patients were evaluable for toxicity (one registered patient never received ATDA). Stomatitis and gastrointestinal toxic effects were predominant but were mild (table 2). A syndrome of chest pain and dyspnea was seen in 12 patients (15%) and caused the hospitalization of five. The etiology of the pain was unclear, but it may have been pericardial in origin (4). It resolved spontaneously and did not recur in those patients who subsequently received further full-dose ATDA. Other drug side effects were unusual. Toxicity did not correlate with duration of treatment or cumulative dose.

Sixty-seven patients who received at least two doses of drug were considered evaluable for response (one patient was ineligible; one had no treatment; six had only one treatment; four lacked tumor measurements; and three had incomplete records). There were three partial responses in the 21 evaluable previously untreated pa-

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tients (14%) and two partial responses in the 46 evaluable previously treated patients (4%). The overall response rate was 7.5% (2.5%–16.6% with 95% confidence). If all patients who received ATDA are considered, the response rate was 6%. Two responses occurred in patients with liver metastases and three in patients with soft tissue masses. The median survival time of all patients from study entry was 36 weeks (48 for those previously untreated and 34 for those with prior chemotherapy).

DISCUSSION

By the usual response criteria, ATDA has only minimal activity against colorectal cancer. However, as shown by the ECOG in phase II trials against colorectal carcinoma, overall survival should also be considered to assess drug activity (3). The 48-week survival of all previously untreated patients is considerably longer than that reported for similar patients treated with standard 5-fluorouracil-containing regimens (3). This difference may be a statistical aberration, but we cannot rule out a drug impact on survival.

TABLE 1.—Patient characteristics*

Characteristic	Previously untreated patients	Previously treated patients	Total
Total entered	28	55	83
Sex			
Male	17	33	50
Female	11	22	33
Median age (yrs)	64	64	64
Performance status (ECOG)			
0–1	24	41	65
2–3	4	14	18
Predominant site of metastases			
Liver	22†	30‡	52
Lung	4	21	25
Soft tissue only	3	10	13

*Unless specified otherwise, values = No. of patients.
†1 with lung.
‡6 with lung.

TABLE 2.—Toxicity*

	No. of patients (%)			
	Grade 1	Grade 2	Grade 3	Total
Anemia	12 (15)	8 (10)	0	20 (25)
Leukopenia	4 (5)	1 (1)	1 (1)	6 (7)
Thrombocytopenia	2 (3)	1 (1)	0	3 (4)
Diarrhea	10 (12)	5 (6)	2 (3)	17 (21)
Stomatitis	14 (17)	10 (12)	2 (3)	26 (32)
Nausea/vomiting	17 (21)	11 (13)	1 (1)	29 (35)
Neuropathy	9 (11)	2 (3)	1 (1)	12 (15)
Chest pain/dyspnea	2 (3)	6 (7)	4 (5)	12 (15)
Hyperuricemia	0	2 (3)	0	2 (3)
Blurred vision	2 (3)	0	0	2 (3)

*ECOG criteria. There was no grade 4 toxicity.

As given in this study, ATDA had minimal toxicity. The dose chosen was based on a phase I trial which did not report toxicity from the use of higher weekly dosage (5). As a result of these findings, the Illinois Cancer Council, under the auspices of the Investigational Drug Branch, National Cancer Institute, is undertaking a phase I trial of ATDA to determine if it is possible to achieve a maximum tolerated dose that is higher than the one previously employed in a weekly schedule. If a higher maximum tolerated dose is found, a further phase II study to define the responsiveness of previously untreated patients with colorectal cancer should be considered.

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BRIEF REPORTS

Hickman Catheter Clots: A Common Occurrence Despite Daily Heparin Flushing[1]

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Central vein thrombosis is the major Hickman catheter complication in our patient population. After noting clots attached to the disposable infusion plug of these catheters, we prospectively determined whether the presence of clots indicated a high-risk group for thrombosis. Forty-three patients (41 with solid tumor, two with acute leukemia) were studied. Catheters were flushed daily with heparin (10 units/ml), and urokinase (5000–10,000 units) was injected when blood could not be drawn. Clots within the catheter lumen were detected visually after expressing, onto a 4 × 4-inch gauze pad, the first 5–10 ml of admixed blood and flush solution or iv infusion fluid drawn from the catheter prior to blood drawing. Clots were detected in 153 of 508 specimens (30%). Forty of 43 patients had clots, varying in length from fragments to 5 cm, in at least one specimen. Inpatients and outpatients had a similar percentage of clotted specimens. Eight patients developed clinical (six) or autopsy-proven (two) thrombosis. There was no difference between patients with and without thrombosis in percent of discards positive for clots. This study indicates that clots are frequently present in the lumina of Hickman catheters. It is likely that these clots embolize to the lungs when catheters are flushed. Consequences of embolization are unknown. [Cancer Treat Rep 71:651–653, 1987]

Central vein thrombosis was our major Hickman catheter complication occurring in eight of 168 solid tumor patients (17%) (submitted for publication). Other investigators have also been concerned by thrombotic complications occurring in patients with external or totally implanted central venous catheters (1,2). Attempts to ameliorate thrombosis have included moderate-dose heparin infusion (3) and low-dose oral warfarin sodium (4).

Because thrombosis is an important cause of morbidity, requiring hospitalization and anticoagulation, and an occasional cause of mortality from pulmonary emboli (5), it is important to identify patients likely to develop thrombosis. In drawing blood from patients with normally functioning Hickman catheters we noted that clots were sometimes attached to the disposable infusion plug. We then prospectively evaluated the occurrence of lumenal Hickman catheter clots to determine whether clot presence indicated a patient population at high risk for thrombosis.

PATIENTS AND METHODS

Forty-three patients, including 16 with lung cancer, 18 with head and neck cancer, four with esophagus cancer, three with colon cancer, and two with acute leukemia, were prospectively studied (table 1). Hickman catheters were in place for 0–17 (median, 3) months. All Hickman catheters were flushed at least daily by nurses (inpatients and clinic visits) or by patients or caretakers (outpatients) with 5 ml of heparin solution (10 units/ml). If blood could not be drawn from a catheter, urokinase (5000 units) was injected into the catheter and left for 30 minutes before blood drawing was again attempted. If unsuccessful, a second dose of urokinase was injected into the catheter and the procedure was repeated.

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TABLE 1.—Hickman catheter clots by cancer histology

Histology	No. of patients	No. of discard specimens	No. of discards positive for clots (%)	No. of patients with thrombosis
Lung				
Adenocarcinoma	5	83	32 (39)	3
Squamous cell carcinoma	8	73	29 (40)	4
Small cell carcinoma	3	18	4 (22)	0
Head and neck	18	234	61 (26)	0
Esophagus	4	58	14 (24)	1
Colon	3	29	10 (34)	0
Acute leukemia	2	13	3 (23)	0
Total	43	508	153	8

Blood for clinical laboratory studies was drawn by a two-syringe method. The first 5–10 ml of admixed blood and flush solution or iv infusion fluid was immediately expressed onto a 4 × 4-inch gauze pad that was visually inspected for clots. The second syringe was then used to obtain a blood specimen for laboratory study.

Clinically manifest central venous thrombosis was diagnosed on the basis of physical findings. Clinical diagnosis was confirmed by venography or autopsy.

RESULTS

Table 1 lists lumenal Hickman catheter clots by tumor histology. Forty of the 43 study patients had clots in at least one discard specimen. Of the three patients without clots (one with squamous cell lung carcinoma and two with head and neck cancer), one had only a single unsuccessful attempt at blood drawing and the other two had five discards examined. Altogether, clots were found in 153 of 508 discard specimens (30%). Clots varied in size from fragments to 5 cm in length. Of the 508 discard specimens, 229 were obtained from inpatients (catheters flushed by nurses) and 279 from outpatients (catheters flushed by patients or caretakers). Of the 153 positive discards, 74 were from inpatients and 79 from outpatients. Twenty-one specimens were obtained from inpatients receiving iv infusions at the time that the discard sample was obtained. None of these had clots. Thrombosis occurred in eight patients, including six with superior vena caval syndrome and two with superior vena caval thrombosis detected only at autopsy. One autopsied patient had bilateral pulmonary emboli. Ninety-nine discards were collected from the above eight patients and 28 (28%) demonstrated clots. Three patients with thrombosis had 20%–25% of all discard specimens positive for clots, four had 26%–50% of discards positive for clots, and one had a clot in the only collected specimen.

DISCUSSION

This study demonstrates the frequent presence of clots in the lumina of Hickman catheters. Forty of the 43 patients (93%) had clots in at least one specimen, and 153 of the total 508 specimens collected (30%) had visible clots up to 5 cm in length. Clots occurred despite daily heparin flushing and intracatheter urokinase when blood could not be drawn. The presence of clots did not, however, predict a high-risk population for development of central venous thrombosis. For the eight patients who developed thrombosis, the percentage of discard specimens revealing clots was similar to that for the 35 patients without clinical thrombosis.

Of greater importance, however, are the possible pulmonary consequences of dislodged Hickman catheter clots. It seems obvious that if clots are sufficiently loosely attached to the Hickman catheter that they can be drawn up into a discard specimen, they are also likely to be dislodged by catheter flushing. Dislodged clots would pass through the right side of the heart and would become trapped in pulmonary circulation. Fibrin or fibrin degradation products released from the clot might then produce pulmonary toxicity apart from the consequences of chronic pulmonary embolization (6). Certain fibrin degradation products damage lung endothelium, causing increased vascular permeability with exudation of plasma proteins and cellular elements into the interstitial space (7,8). Cancer patients frequently develop distressing pulmonary symptoms and often demonstrate chest x-ray abnormalities of uncertain etiology. Whether intralumenal Hickman catheter clots contribute to these symptoms is unknown. This study indicates the scope of intralumenal thrombosis within Hickman catheters. Reliable methods to prevent thrombosis are urgently needed.

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Treatment of Favorable-Prognosis Nonseminomatous Testicular Germ Cell Tumors With Etoposide, Cisplatin, and Reduced Dose of Bleomycin[1,2]

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We have explored a dose reduction of bleomycin in combination with etoposide and cisplatin in the treatment of a good-prognosis subgroup of patients with nonseminomatous germ cell tumors. Twenty-two patients were treated with etoposide, cisplatin, and low-dose bleomycin. All patients achieved complete remission, but four relapsed. Three patients achieved long-term remission after salvage therapy. The disease-free survival rate of 82% (18 of 22 patients) at a median follow-up of 24 months (range, 17–33) was significantly worse compared to the 99% disease-free survival rate in 91 patients receiving full-dose bleomycin, etoposide, and cisplatin. These results are similar to a previously reported 82% disease-free survival rate of 17 patients treated with etoposide and cisplatin alone. The combined results of the 82% disease-free survival rate in the total group of 39 patients treated with etoposide and cisplatin and etoposide, cisplatin, and low-dose bleomycin suggest that the reduced bleomycin regimens in their present schedule constitute inadequate therapy. [Cancer Treat Rep 71:655–656, 1987]

In an attempt to reduce the toxicity of chemotherapy in patients with favorable-prognosis testicular teratoma, we have used regimens in which bleomycin was either omitted or used in a reduced dose. Seventeen patients were treated with etoposide and cisplatin (EP) (1). More recently, 22 patients were treated with bleomycin, etoposide, and cisplatin (B_1EP), with bleomycin (30 mg) given at 3-week intervals instead of weekly (2). We report our experience with this regimen and update EP results.

PATIENTS AND METHODS

Twenty-two patients had histologically confirmed nonseminomatous germ cell tumor and full staging investigations (3). The Royal Marsden Hospital staging classification was used (4). All patients had small-volume disease with good and intermediate prognostic features according to Medical Research Council (MRC) criteria (5). The following stage categories were included: IIA (nine patients); IIB (three); IIIA (one); IIIB (one); and IVO/A/BL_1 (seven). One patient had normal staging investigations. Treatment was given after complete excision of scrotal recurrence. Four patients had high marker levels on MRC criteria (5) (β-human chorionic gonadatropin $\geq$ 1000 IU/L and/or α-fetoprotein $\geq$ 500 IU/L), while 18 patients had low or undetectable levels.

Chemotherapy consisted of cisplatin (20 mg/m^2/day) on Days 1–5, etoposide [120 mg/m^2/day (maximum, 200 mg)] on Days 1–3, and a bolus iv injection of bleomycin (30 mg) only on Day 2 of each cycle. Four cycles of chemotherapy were given at 3-week intervals. Residual masses were excised after chemotherapy.

RESULTS

Eighteen of 22 patients treated with B_1EP are alive and disease-free after initial therapy, with a median follow-up of 24 months (range, 17–33). All 22 patients achieved complete remission (CR): 16 after chemotherapy alone and an additional six after surgical excision of residual masses. Four patients relapsed and three of these are currently alive and disease-free after salvage treatment. One patient died of progressive intra-

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abdominal embryonal sarcoma arising from a para-aortic node. One patient died 26 months after orchidectomy following myocardial infarction. At last follow-up in the same month he was free of disease. Overall, 20 patients (91%) are alive and disease-free.

Detailed results of EP alone have been reported by Peckham et al (1). Currently, 14 of 17 patients (82%) are alive without evidence of disease, with a median follow-up of 44 months (range, 39–49). All three relapsed patients died.

Toxicity

Only one patient had one cycle of treatment delayed. Eight patients had hemoglobin levels < 10 g/dl, ten had total wbc counts $< 2 \times 10^9$/L (no septicemic episodes), and eight had platelet counts $< 100 \times 10^9$/L. Three patients had transient sensory peripheral neuropathy and two of these had transient Lhermitte's phenomena. One patient had a reduction of glomerular filtration rate to < 80 ml/minute.

One patient had intermittent chest pain associated with bleomycin administration and one developed Raynaud's syndrome. There was no bleomycin-related functional or radiological lung toxicity and no significant skin or sc inflammation.

DISCUSSION

The best results in the treatment of metastatic teratoma are obtained in patients with small-volume disease (5). The disease-free survival rate of 91 patients with small-volume disease treated at The Royal Marsden Hospital with bleomycin, etoposide, and cisplatin or cisplatin, vinblastine, and bleomycin between 1976 and 1986 is 99% (90 of 91 patients) (3).

We attempted to reduce the toxicity in this group of patients by reducing or omitting the bleomycin dose. An initial study of EP alone demonstrated an 82% long-term disease-free survival rate (1). The addition of low-dose bleomycin (30 mg once every 3 weeks) has resulted in the same disease-free survival rate of 82%. This represents a significantly worse result than standard bleomycin, etoposide, and cisplatin chemotherapy ($P < 0.05$). Preliminary results of a randomized trial from Australia comparing cisplatin, vinblastine, and bleomycin versus cisplatin and vinblastine alone showed relapse rates of 5% (two relapses among 41 patients) and 9% (four relapses among 44 patients), respectively (6). The recurrence rates of "good-risk" patients randomized between EP and vinblastine, dactinomycin, cyclophosphamide, bleomycin, and cisplatin (VAB-6) at the Memorial Sloan-Kettering Cancer Center were reported as 18% (nine relapses among 50 patients) and 9% (five relapses among 55 patients), respectively (7).

Four patients relapsing from CR did so at the site of previous disease. Two patients had residual abnormalities in the para-aortic region measuring < 1 cm and not amenable to surgical excision. Because an upper limit of 200 mg was applied to the daily etoposide dose, patients with body surface areas > 1.67 m^2 did not receive the full recommended dose of 120 mg/m^2/day. The relapse rates in patients receiving < 100, 100–111, and 120 mg/m^2/day were 33% (three relapses among nine patients), 20% (four relapses among 20 patients), and 0% (no relapses among seven patients), respectively ($P = 0.1$; test for trend). These data are consistent with a dose-response relationship for etoposide.

We conclude that in the schedules described above the dose reduction of bleomycin was associated with an increased recurrence rate. This may not occur with higher etoposide doses and the current European Organization for Research and Treatment of Cancer study of EP with a 5-day etoposide schedule may elucidate this.

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Phase II Trial of Idarubicin in Patients With Pancreatic Cancer[1]

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One of the new-generation derivatives of doxorubicin is idarubicin, which lacks the methoxy group in position 4 of the aglycone (1). Idarubicin was entered in clinical trials based on its activity against a number of tumor systems (2) and its observed decrease in cardiotoxicity when compared with daunorubicin in tumor models (3). The dose-limiting toxic effect in a phase I trial was myelosuppression.

Results of a phase II trial of idarubicin in patients with measurable pancreatic cancer are reported here. An iv dose of 15 mg/m^2 was initiated every 3 weeks (4) as shown by phase I studies.

MATERIALS AND METHODS

Thirty-eight patients with measurable disease and histologically confirmed pancreatic carcinoma were entered. Criteria for entry were Karnofsky performance status > 50%, serum bilirubin < 1.5 mg/dl, wbc count > 3500/mm^3, platelet count > 50,000/mm^3, and signed informed consent. The drug was given iv every 3 weeks and doses were escalated or de-escalated by 2.5-mg/m^2 increments depending on wbc count nadirs.

The initial starting dose was 15 mg/m^2; two patients who were heavily pretreated started therapy at 10 mg/m^2.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	
Entered	38
Evaluable	32
Median age in yrs (range): 60.5 (37-74)	
Median Karnofsky performance status (range): 70% (50%-90%)	
Sex	
Male	15
Female	23
Prior therapy	
None	28 (75)
Chemotherapy	6 (15)
Radiation therapy	2 (5)
Chemotherapy and radiation therapy	2 (5)
Histologic diagnosis	
Adenocarcinoma	36 (94)
Anaplastic carcinoma	1 (3)
Epidermoid carcinoma	1 (3)
Response	
Complete	0
Partial	2
Stable disease	4
Disease progression	26

RESULTS

Of the 38 patients entered in the study (table 1), 32 were evaluable for response. All patients were evaluable for toxic effects. One patient withdrew from the study and one patient died before the completion of her second cycle. Four patients died within the first 2 weeks of therapy due to disease progression. Postmortem examination showed multiple pulmonary emboli and pneumonia. There were no complete responses and two patients achieved partial response for 3 months. Both of these responses were determined by repeated computerized tomographic scans and were observed in previously untreated patients. Twenty-four patients had disease progression and four had stable disease from 83 to 134 days. One of the responding patients died from nadir sepsis (wbc count, 0.5/mm^3).

Gastrointestinal toxic effects and fatigue were difficult to evaluate in the patient population because of their disease-induced symptoms. Myelosuppression was the prominent toxic effect; median wbc count nadir was 1900/mm^3 (range, 0–4000) at Day 10, with recovery by Day 14, and median platelet count nadir was 91,000/mm^3 (range, 18,000–273,000). Profound thrombocytopenia was observed in seven patients who had had combination

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chemotherapy containing mitomycin. Five patients were evaluated by sequential radionuclide cardioangiography. There was no change in four patients who received a total dose of 60 mg/m^2. One patient who received a total of 69.5 mg/m^2 had a 7% decrease in the resting left ventricular ejection fraction.

DISCUSSION

Thirty-eight patients, 28 of whom were previously untreated, were entered in this phase II trial. The main toxic effect found was myelosuppression. There were no complete responses; two partial responses of short duration were achieved. In addition, three patients died from nadir sepsis. The 95% confidence interval was 0%–8% for the entire group and 0%–8% for the previously untreated patients. These data indicate that idarubicin appears to be more toxic and less active than doxorubicin in the treatment of pancreatic cancer.

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Treatment of Refractory Non-Hodgkin's Lymphomas of Unfavorable Histology With Teniposide, Cytarabine, and Cisplatin[1,2]

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Non-Hodgkin's lymphomas of intermediate- and high-grade histologies are responsive to combination chemotherapy (1). However, because relapses remain common, the need for effective second-line therapy is evident. Three agents not used in most front-line regimens appear promising: teniposide (VM-26), cytarabine (Ara-C), and cisplatin (2–4). VM-26 was investigated because its combination with Ara-C demonstrates high clinical efficacy for recurrent childhood lymphocytic leukemia (3). Synergy between cisplatin and Ara-C or VM-26 has been reported (5).

PATIENTS AND METHODS

Twenty-four patients with refractory lymphoma were entered in this study. Patient characteristics are shown in table 1. The outpatient regimen consisted of VM-26 at a dose of 60 mg/m^2 infused iv over 30 minutes daily for 3 days, Ara-C—45 mg/m^2 every 12 hours for six doses given alternately by iv or sc injection, and cisplatin—60 mg/m^2 infused over 30 minutes on Day 1. Patients had blood cell counts, creatinine clearances, and magnesium levels performed every 3 weeks. Standard criteria were used to assess response.

RESULTS

Two of 24 patients (8%) achieved complete response (CR) of 4 and 37+ months' duration. Both patients had progressive disease at study entry. Nine patients achieved partial response (PR) (38%), with a median duration of 4 months (range, 2–22). Two had high-grade lymphomas and previously achieved CR lasting 10 and 31 months. Ten patients with high-grade lymphoma achieved two CRs and five PRs versus 14 patients with intermediate-grade lymphoma who achieved four PRs.

The major toxic effect was myelosuppression, but there were no treatment-related deaths.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	24
Evaluable	24
Median age in yrs (range): 44 (24–74)	
Histology (grade)	
Intermediate	14
High	10
B symptoms	11
Sites of disease at relapse	
Nodes	15
Bone marrow	6
Spleen	4
Bone	4
Gastrointestinal	3
Soft tissue	3
CNS (meningeal)	3
Prior therapy	
Chemotherapy regimen*	
CHOP/B-CHOP	13
M-BACOD	8
COP-BLAM	2
PROMACE-MOPP	1
Radiation therapy	13
Response to primary regimen	4
Resistance to primary regimen	20
Median No. cycles of study regimen (range): 2 (1–5)	

continued

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TABLE 1.—*continued*

Characteristic	No. of patients (%)
Median survival in mos (range): 6 (1–41+)	
Toxic effects	
Nausea	24 (100)
Alopecia	8 (33)
Flushing/hives/fever	6 (25)
Wbc count < 1000/mm^3	6 (25)
Hct < 25 mg/100 ml	6 (25)
Platelet count < 50,000/mm^3	5 (25)
Hearing loss	3 (15)
Response	
CR	2/24 (8)
PR	9/24 (38)
None	13/24 (54)

*CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; B-CHOP = bleomycin-cyclophosphamide, doxorubicin, vincristine, and prednisone; M-BACOD = methotrexate-leucovorin, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone; COP-BLAM = cyclophosphamide, vincristine, and prednisone or prednisolone-bleomycin, procarbazine, and dexamethasone; and PROMACE-MOPP = doxorubicin, cyclophosphamide, methotrexate, prednisone, and etoposide-mechlorethamine, vincristine, procarbazine, and prednisone.

DISCUSSION

The teniposide, cytarabine, and cisplatin regimen achieved an overall response rate of 46%, which is comparable to other series (1). It was most effective in patients with refractory high-grade lymphomas; however, only one durable CR was achieved.

We conclude that this regimen deserves further testing in refractory high-grade lymphomas.

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Phase II Trial of Etoposide in the Management of Advanced or Recurrent Mixed Mesodermal Sarcomas of the Uterus: A Gynecologic Oncology Group Study[1,2]

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In 1977, members of the Gynecologic Oncology Group (GOG) initiated a series of phase II trials designed to test new agents in a number of different advanced or recurrent gynecologic malignancies, including mixed mesodermal sarcomas of the uterus.

Etoposide was chosen for the current trial because of its activity in several nongynecologic tumors; it was also noted to have minimal activity against ovarian adenocarcinoma (1). It was given at a dose of 100 mg/m^2 diluted in 150 ml of normal saline and administered over a 45-minute period on Days 1, 3, and 5. The cycle was repeated every 28 days. A complete description of materials and methods is contained in an earlier paper (1).

Complete response was defined as the disappearance of all gross evidence of disease for at least 4 weeks. Partial response was defined as a $\geq$ 50% reduction in the product of the largest diameter and its perpendicular of each measurable lesion for a period of at least 4 weeks. Disease progression was defined as a $\geq$ 50% increase in the product of the largest diameter and its perpendicular of any lesion documented within 8 weeks of study entry or the appearance of any new lesion within 8 weeks of entry in the study. Stable disease was defined as any disease not meeting any of the above criteria.

Between April 1978 and November 1985, 31 evaluable patients were accrued. Patient characteristics, response to therapy, and toxic effects are shown in table 1. Two partial responses lasting 2 and 6 months were noted; 15 patients had stable disease, while 14 had disease progression. There were no complete responses. Of the two partial responders, one had previously received doxorubicin followed by radiotherapy; the other had received

TABLE 1.—Patient characteristics and results

Characteristic	No. of patients
Total evaluated	31
Median age in yrs (range): 65 (46–84)	
GOG performance status	
0	10
1	7
2	4
Site of disease	
Pelvis	19
Outside pelvis	12
Prior surgery	
TAH/BSO*	25
Laparotomy and biopsy	4
None	2
Prior radiotherapy	14
Prior chemotherapy	29
Median No. of courses (range): 2.0 (1–15)	

*TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy.

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21 courses of vincristine, doxorubicin, and cyclophosphamide and had not been treated with radiation therapy.

Leukopenia was the most serious toxic effect and was observed in 22 of the 31 patients, with a median wbc count nadir of 2600/mm^3 (range, 500–3900). Thrombocytopenia was noted in seven patients, with a median platelet count nadir of 104,000/mm^3 (range, 52,000–140,000). Fourteen patients had mild to moderate nausea and vomiting.

At the dose and schedule tested, etoposide appears to have minimal activity in the treatment of advanced or recurrent mixed mesodermal sarcomas of the uterus.

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Phase II Trial of Ifosfamide and Mesna in Advanced Colorectal Cancer[1,2]

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Ifosfamide (IFX), a member of the active oxazaphosphorines, has attracted clinical interest because of its lack of cross-resistance with cyclophosphamide in preclinical testing and suggested activity in solid tumors. Dose escalation has been limited by hemorrhagic cystitis (1); however, with the use of mesna, a uroepithelial protective agent (2), and a fractionated schedule of IFX over 5 days (3), this toxicity has been reduced. Demonstrated activity in transplantable murine colon tumors 26 and 38 (4) initiated a phase II trial of IFX and mesna in patients with metastatic colorectal carcinoma.

METHODS

All patients with advanced colorectal carcinoma, pathologically confirmed at the Memorial Sloan-Kettering Cancer Center, were eligible if the following criteria were met: no prior chemotherapy; Karnofsky performance status > 60; wbc count > 4000/mm^3; platelet count > 150,000/mm^3; serum bilirubin < 2.0 mg/100 ml; serum creatinine < 1.5 mg/100 ml; creatinine clearance > 50 ml/minute; urine sediment with < 5 rbcs/high-power field; and bidimensionally measurable disease which was not previously irradiated (table 1).

The dose of IFX was 1.5 g/m^2/day for 5 consecutive days each month. Mesna at 30% of the IFX dose was given iv before IFX and repeated twice at 4-hour intervals after each IFX dose. In patients with no toxic effects, dose was escalated by 0.25 g/m^2/day. Dose was attenuated by 25% of the previous dose for patients with hematuria (> 11 rbcs/high-power field), wbc count < 1900/mm^3, platelet count < 50,000/mm^3, and/or increase in creatinine > 0.5 mg/100 ml. Serum electrolytes and urinalyses were repeated daily during treatment and serum creatinine was done every other day to monitor toxic effects. Reassessment of measurable disease was done after two cycles of therapy, using standard definitions of response. Therapy was continued until disease progression or unacceptable toxic effects as defined by the World Health Organization (WHO) scale (5).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total entered	21
Age (yrs)	
< 60	10
60-70	8
> 70	3
Male	13
Female	8
Performance status*	
70	7
80	5
90	9
Previous treatment	
Chemotherapy	0
Radiotherapy	4
No. of cycles	
≥ 2	15
< 2 with disease progression	3
< 1	3
Dose escalation	6
Dose attenuation	4
Site of disease	
Liver	19
Lung	5
Intra-abdominal	2
Nodes	7
Pelvis	1

*Karnofsky performance scale.

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TABLE 2.—Toxicity

Toxic effect	Modified WHO scale 1	2	3	4	No. of patients
Nausea/vomiting	5	7	—	—	12
Wbc count nadir	—	—	8	1	9
Alopecia	—	1	5	1	7
Diarrhea	4	1	—	—	5
Hematuria	3	—	1	—	4

RESULTS

There were no clinical responses among 18 evaluable patients; two patients had a > 25% decrease in carcinoembryonic antigen levels; and six patients experienced stable disease for a median duration of 4.1 months (range, 2.1–4.6).

Toxic effects are shown in table 2. Hematologic toxicity included a granulocyte count nadir < 2000/mm^3 in nine patients, of whom two had wbc counts < 1000/mm^3, which resulted in hospitalization. No thrombocytopenia, stomatitis, or biochemical abnormalities were observed. Neurotoxicity was not seen except for transient confusion in one patient.

DISCUSSION

Despite moderate hematologic toxicity, no objective responses were seen among 18 previously untreated patients with colorectal carcinoma, with a 95% confidence interval of 0%–18.5%. We conclude that IFX has no significant activity in patients with advanced colorectal carcinoma at this dose and schedule.

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Synthetic Enkephalin Analog in the Treatment of Cancer Chemotherapy-Induced Vomiting

Vomiting is one of the major side effects of cancer chemotherapy. The mechanisms by which cytotoxic drugs cause vomiting have not yet been clarified. Some experimental studies would seem to suggest altered endogenous opioid peptide secretion in cancer chemotherapy-induced vomiting (1-3). Gros et al (1) observed decreased levels of enkephalins in the mouse brain treated with cytotoxic drugs. Moreover, it has been shown that antiemetic effects of cannabinoids in preventing chemotherapy-induced vomiting are blocked by the administration of an opioid antagonist, such as naloxone (2). Despite these interesting results, no data are available on the possible use of opioid peptides in the treatment of cancer chemotherapy-induced vomiting.

Based on the fact that chemotherapy-induced vomiting could be due to damage of the endogenous opioid system, we have carried out a pilot study to evaluate the effectiveness of a longer-acting met-enkephalin analog (FK 33-824) in treating cancer chemotherapy-induced vomiting.

MATERIALS AND METHODS

The study was carried out in 14 informed cancer patients. Each patient was used as his own control. In a single blind, cross-over study, patients were treated with an iv infusion of FK 33-824 at a dose of 0.3 mg in 20 ml of saline solution over 10 minutes or with saline alone as placebo before receiving chemotherapy. No antiemetic drugs were prescribed during the study. The emetic symptoms were graded as follows: 0 = no nausea or vomiting; 1 = nausea only; 2 = one to two episodes of vomiting during the day; 3 = three to five episodes during the day; 4 = six to ten episodes during the day; and 5 = more than ten episodes during the day or vomiting protracted for > 1 day. Data were reported as mean ± SD, and results were analyzed by Student's t test. Clinical data of cancer patients and the individual vomiting scores are shown in table 1.

All patients reported an unpleasant feeling of heaviness in the body after FK 33-824. No other undesirable side effects were observed.

RESULTS

By considering overall patients treated with different chemotherapeutic regimens, no significant differences were seen between "placebo" and "treated" mean values of vomiting scores (2.7 ± 1.2 vs 1.6 ± 1.0; not significant). On the contrary, among those patients who received combinations containing cisplatin, in three of seven patients (Nos. 2, 5, and 7) FK 33-824 completely

TABLE 1.—Clinical data of cancer patients and individual vomiting score*

Patient No.	Sex	Age (yrs)	Tumor	TNM	Previous treatment	Chemotherapy	Vomiting score	
							Placebo	FK 33-824
1	M	57	Lung	T2N2M1	None	CDDP/VP-16	4	2
2	F	54	Lung	T2N2M1	RT	CDDP/VP-16	5	0
3	M	56	Lung	T2N1M0	RT	CDDP/VP-16	4	2
4	M	73	Lung	T2N1M0	None	CDDP/VP-16	4	2
5	F	59	Lung	T2N1M0	RT	CDDP/VP-16	3	0
6	F	42	Uterine cervix	T2N0M0	None	CDDP	3	3
7	F	59	Uterine cervix	T3N1M0	None	CDDP	3	0
8	F	48	Lung	T3N1M0	None	CEV	2	3
9	F	49	Breast	T3N2M1	None	FEC	1	2
10	F	36	Breast	T2N1M0	Surgery	CMF	1	1
11	F	53	Breast	TxNxM1	Surgery	CMF	2	2
12	F	44	Breast	T2N1M0	Surgery	CMF	2	2
13	F	31	Breast	T4N3M1	None	FEC	2	2
14	F	56	Breast	T2N1M0	Surgery	CMF	2	2

*RT = radiotherapy; CDDP/VP-16 = cisplatin (20 mg/m^2 iv on Days 1-5) + etoposide (100 mg/m^2 iv on Days 1-3); CDDP = cisplatin (1 mg/kg of body weight/wk); CEV = cyclophosphamide (750 mg/m^2), epirubicin (50 mg/m^2), and vincristine (1 mg/m^2 iv on Day 1); FEC = 5-fluorouracil (500 mg/m^2 on Days 1 and 8), epirubicin (50 mg/m^2) and cyclophosphamide (500 mg/m^2 iv on Day 1); and CMF = cyclophosphamide (600 mg/m^2), methotrexate (40 mg/m^2), and 5-fluorouracil (600 mg/m^2 iv on Day 1).

blocked chemotherapy-induced vomiting and nausea, while after placebo alone they vomited at least three times during the day. Three other patients (Nos. 1, 3, and 4) reported an evident amelioration with FK 33-824. In one patient only (No. 6), FK 33-824 in saline solution was unable to prevent cisplatin-induced vomiting. The difference between mean values of vomiting scores observed after FK 33-824 and those after placebo was statistically significant (1.3 ± 1.2 vs 3.7 ± 0.7; $P < 0.01$).

In contrast to the significant benefits observed after FK 33-824 in patients treated with cisplatin, no effects (Patient 13) or more intense vomiting (Nos. 8 and 9) were seen in patients receiving therapy with chemotherapeutic regimens containing epirubicin. Because of the small number of patients, however, it was not possible to make statistical analyses. Finally, the emetic symptoms were the same after placebo or FK 33-824 in the last four patients treated with CMF (cyclophosphamide, methotrexate, and 5-fluorouracil).

Even though these results must be confirmed in a greater number of patients, they suggest that FK 33-824 might be effective in the treatment of vomiting induced by cisplatin, and they support the hypothesis that opioids may be involved in cancer chemotherapy-induced emesis. The low effectiveness of FK 33-824 in some patients could be due to its brief half-life, to an inadequate dose, or to doses > 0.3 mg; a more prolonged treatment during the day might be needed in nonresponding patients.

The mechanisms by which FK 33-824 may affect chemotherapy-induced vomiting are difficult to explain, but it is possible to propose that it is mediated via enkephalin pathways. Moreover, the opposite effects determined by FK 33-824 in preventing vomiting induced by cisplatin or by epirubicin seem to suggest that these two cytotoxic drugs could exert different actions on the endogenous opioid system. In fact, in our previous studies (unpublished data), we observed that cisplatin, but not doxorubicin or cyclophosphamide, determined a significant decrease in β-endorphin plasma levels. Alternatively, it is possible to hypothesize the existence of different vomiting receptors for different chemotherapeutic agents.

In conclusion, this study states the paradox that FK 33-824 can both cause vomiting and treat chemotherapy-induced emesis. This implies that opioid pathways are involved in both processes, through mechanisms that are as yet undetermined. Further investigations, by using different opioid peptides and/or opioid antagonists (eg, naloxone or naltrexone), will be needed to clarify the role played by the endogenous opioid system in cancer chemotherapy-induced vomiting and to evaluate their effectiveness in its treatment.

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"Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?"

The review "Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?" serves to illustrate several additional considerations. These considerations are especially relevant to the development of treatment for a disease which is sometimes cured with chemotherapy and suggest possible missed opportunities in drug development strategies.

Point 1.—Hexamethylmelamine (HMM) was reviewed without requiring thorough analysis of its possible impact on patients with optimum prognostic characteristics. This may result in false-negative expectations and comparisons.

There is some likelihood of false-negative studies of primary chemotherapy as a consequence of ignoring prognostic factors (1). For patients with both an optimum small volume of disease and good performance status, combinations produce high rates of response, even after primary therapy fails (2).

Too few patients have had HMM as single-agent primary therapy after optimum surgery in order to apply trial criteria requiring long-term clinical disease-free survivors. Complete responders, not cited in the review, exist (3). Long-term complete responders to HMM-containing combinations are probably not reported due to short follow-up times in many reported studies rather than their nonexistence.

The constraints of a small sample size are compounded

in evaluating treatment of optimum patients. HMM or any of the other "active," equally poorly tested drugs might improve the 5-year survival by 10%, possibly 20%, effectively doubling true 5-year survival. Such improvements might remain undetected by current methods of trial and analysis, yet would benefit some 2000 additional patients a year in the United States.

The review makes a valuable contribution in thoroughly documenting the shortcomings of conventional combinations as treatment for patients with initially poor prognostic characteristics or late diagnosis of primary treatment failure. It does not deal with curing or substantially palliating optimum patients. One can make a favorable case for monitoring this drug in a large registry study of a combination and, where possible, performing a meta-analysis.

Point 2.—HMM is present in combinations which produce or equal best cross-trial results, some with curative implications. HMM may be more than a needle in a haystack. A combination which reproduces "best results" can be a "magic bullet."

CHAD (cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin) produced significant improvements in both response rate and progression-free survival versus melphalan in the Eastern Cooperative Oncology Group phase III study as did CHAP-5 (cyclophosphamide, HMM, doxorubicin, and cisplatin) versus HexaCAF (HMM, cyclophosphamide, 5-fluorouracil, and methotrexate) in surgically proven response, progression-free interval, and survival in the Netherland phase III study. In phase II trials the Vanderbilt study of H-CAP (HMM, cyclophosphamide, doxorubicin, and cisplatin) produced best surgically proven response rates for optimally debulked patients (4). Arizona (5) and Mount Sinai (6) studies describe high-quality responses and survival times, some responses benefiting patients after multiple drugs failed.

Point 3.—HMM is inadequately studied in the laboratory. As a fundamental step in drug development, an optimum combination remains to be characterized in preclinical studies.

Information supports the existence of alkylating agent-HMM synergism (7), yet these drugs are often separated or compromised in concocting combinations which failed. The virtual absence of relevant references to laboratory studies of drug interaction illustrates this deficiency.

Point 4.—HMM appears to be the equal of the three major drugs in current usage in all substitution studies which "almost isolate HMM." Finding that one drug can be substituted for another may provide indirect evidence of efficacy. It is not necessarily evidence of equivalency. The additional drug, such as HMM, may possibly be employed effectively as part of an elective cross-over regimen or with dosage intensification. Patients may also benefit from pharmacologic or toxicologic differences; eg, some may need drugs such as HMM which produce less than average bone marrow suppression or which are available in an oral formulation.

The Milan trial found equivalency of HMM and cisplatin [PAC (cisplatin, doxorubicin, and cyclophosphamide) versus HAC (HMM, doxorubicin, and cyclophosphamide)]. Recognizing the limitations of cross-trial analyses, median survival was substantially better than that found by the Gynecological Oncology Group with AC (doxorubicin and cyclophosphamide) alone. The Yale study compared doxorubicin to HMM [HC (HMM and cyclophosphamide) vs AC]. The Central Pennsylvania Group compared HMM and cytarabine [HAP (HMM, doxorubicin, and cisplatin) vs CAP (cyclophosphamide, doxorubicin, and cisplatin)]. In addition, the Mayo Clinic (8) and the Netherland studies[1] also found HCAP (HMM, cyclophosphamide, doxorubicin, and cisplatin) and CHAP-5, respectively, equivalent to high-dose CP (cyclophosphamide and cisplatin).

Thoroughness requires tests of a combination, which meets good preclinical test criteria, in at least three distinct groups of patients with good performance status and minimum volume of residual disease. In the case of ovarian cancer, these groups include patients treated as follows: (*a*) immediately after successful optimum primary debulking surgery; (*b*) in elective sequence after achieving minimum disease status, not necessarily complete remissions; and (*c*) upon earlier diagnosis of primary chemotherapy failure.

There are so few equally good candidate drugs for trials that one cannot justify abandoning a non-cross-resistant, relatively marrow-sparing drug with a possible unique mechanism of action and clear activity. In the case of ovarian cancer, routine drug trial evaluation criteria are not enough. Additional efforts deserve to be focused on studies and strategies which have the objective of substantial palliation or cure.

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Reply

We express our gratitude to Dr. Bruckner for his comments regarding the publication entitled "Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?" (1) We especially appreciate the importance of Point 3 regarding the absence of preclinical information which might indicate the optimal hexamethylmelamine (HMM)-containing combination of drugs and their sequencing. The lack of this information is likely due to the difficulty in performing preclinical HMM activity studies because metabolic activation is required (2); indeed, HMM is inactive in most murine tumor models (3).

It should be pointed out that the conclusion made in Point 4 might be strengthened if: (*a*) in each trial where a comparison of HMM was being made to another drug both HMM and the other drug were given in an optimum manner; (*b*) the other constituent(s) of the combination were exactly the same in each arm; and (*c*) patient numbers were sufficient to allow conclusions regarding activity to be made with an acceptable degree of certainty. Only (*b*) was satisfied by the published trial results that "almost isolate HMM."

Regarding Point 2, the presence of a particular drug in an active combination obviously does not provide information concerning that drug's contribution to the combination's activity. Although it may not always be necessary to define each component relative to the total combination, it is extremely useful to have some knowledge of the relative efficacy/toxicity contribution of each drug to the treatment of each specific disease.

Regarding Point 1, subgroup analysis was not always reported in the clinical trials cited (1); therefore, subset analysis was not reported routinely in the review. However, the studies described by Dr. Bruckner for patient subsets will still not provide knowledge of the activity of HMM unless: (*a*) a question regarding the contribution of HMM is asked in the trial design; and (*b*) the required number of patients are accrued and the results of the trial(s) published.

Finally, the title of the manuscript does not imply relative usefulness of HMM, rather it is intended to reflect our lack of knowledge regarding the role of this drug after > 15 years of clinical trials.

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Drug Eruption Localized to Previously Irradiated Skin

The phenomenon of radiation recall is most commonly associated with doxorubicin (1) and dactinomycin (2). Other chemotherapeutic agents such as methotrexate, bleomycin, cyclophosphamide, and 5-fluorouracil are also capable of causing skin reactions in previously irradiated areas (1). Drugs other than chemotherapeutic agents can also occasionally cause radiation recall reactions. Shelley et al (3) reported two cases of drug eruptions that were limited to sites of previous radiation damage. One patient developed radiodermatitis due to electron-beam therapy, and 7 weeks later a codeine-related skin reaction occurred that was confined to the area of the radiodermatitis. The second case was a patient who sustained severe sunburn and 7 months later developed a toxic epidermal necrolysis reaction to trimethoprim-sulfamethoxazole limited to the exact site of the previous bullous sunburn. We report a patient who developed a drug eruption secondary to trimetho-

[1] NEIJT PJ, BOKKEL HUININK WWT, VAN DE BURG MEL, ET AL. Randomized trial comparing two combination chemotherapy regimens (CHAP-5 vs CP) in advanced ovarian carcinoma. Manuscript submitted to the *Journal of Clinical Oncology*.

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prim-sulfamethoxazole limited to an area of prior therapeutic irradiation.

The patient is a 45-year-old white woman with a history of stage II breast cancer diagnosed in 1982. She had a modified radical mastectomy and 1 year of adjuvant chemotherapy. The patient did well until December 1984 when she developed recurrent cancer in the mastectomy site. She was treated with 5000 rad to the right chest wall. In September 1986 she developed multiple metastatic lung nodules. She was admitted for evaluation prior to chemotherapy and was found to have a urinary tract infection which was treated with trimethoprim-sulfamethoxazole. Three days later she developed an eruption localized to the area of prior radiation. Physical examination showed an erythematous, edematous area of the right chest wall with remarkable sharp margination between the area of dermatitis and normal skin. The margins correlated exactly with the edges of the radiation portal. A skin biopsy revealed dermal edema with a perivascular lymphohistiocytic infiltrate in the upper dermis. Trimethoprim-sulfamethoxazole was discontinued, and the skin eruption improved slowly over the next few weeks. The patient was later treated with the VATH (vinblastine, doxorubicin, thiotepa, and fluoxymesterone) chemotherapy regimen.

The term "radiation recall" is used when an acute skin reaction mimics the original radiation dermatitis. It has been reported to occur secondary to a number of antitumor agents and a few other drugs (1-4). Our patient had a radiation recall reaction to trimethoprim-sulfamethoxazole which could have been confused with such a reaction to a cancer chemotherapeutic agent, if she had been receiving such treatment. When recall dermatitis occurs in patients with cancer, the antitumor agent in use may not always be the initiator of the reaction. One must consider the possibility that other drugs being administered such as codeine and trimethoprim-sulfamethoxazole may have precipitated the radiation recall.

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High-Dose (200 mg/m^2) Cisplatin-Induced Neurotoxicity in Primary Advanced Ovarian Cancer Patients

To date, quite a few controversial data are available on high-dose cisplatin (hd-cddp)-induced toxicity. Peripheral neuropathy was first described by Ozols and Young (1) as the dose-limiting factor both in refractory and untreated ovarian cancer patients.

On the other hand, Gandara et al (2) recently observed myelosuppression as the major side effect in a small series of advanced non-small cell lung cancer patients. According to Litterst (3), all of these investigators (1,2) demonstrated protection against hd-cddp-induced nephrotoxicity with hypertonic saline as the vehicle of administration and used large continuous-hydration volumes. We are carrying out a pilot study to evaluate single-agent hd-cddp efficacy and toxicity in advanced untreated ovarian carcinoma.

Our administration schedule was similar to that previously described by Ozols and Young (1): cddp at a dose of 40 mg/day for 5 days in hypertonic saline (100 mEq of NaCl/200 ml of normal saline in a 2-hour infusion) with 6 L/day of normal saline plus 120 mEq of KCl/day and 10 mEq of CaMgCl/day. Intensive hydration started 12 hours before the first dose of cddp and continued until 12 hours after the last dose on Day 5; iv mannitol with or without furosemide was routinely used daily for an enhanced diuresis, and hd-cddp was repeated every 28 days for three courses. To date, a total of 47 cycles have been administered to the 18 patients included in the study. The median age is 54 years (range, 28-70).

As in previous observations (1,2,4), renal toxicity was mild. Only three of 18 patients had grade I nephrotoxicity (1.26-2.5 $\times$ baseline serum creatinine).

Bone marrow toxicity was acceptable, with only one of 18 patients showing grade III leukopenia. Grade I-II myelosuppression was, however, detected in the remaining 17 patients. No bleeding or chemotherapy-related infection was observed and myelotoxicity did not appear to be cumulative. On the other hand, 13 of 16 patients who received more than two courses developed peripheral neuropathy, confirmed by neurological examination, electromyography, and sequential evoked poten-

TABLE 1.—hd-cddp-induced neurotoxicity*

No. of courses	No. of patients	Time after last course (mos)	No. of patients with neurotoxicity		
			Grade I	Grade II	Grade III
3	5	6	—	2	3
	3	3	2	1	—
	5	1	3	1	—
2	3	1	1	—	—
1	2	1	—	—	—

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tial, performed before hd-cddp, repeated monthly during hd-cddp and every 3 months thereafter (table 1). Grade III neurotoxicity with gait disturbances was detected in three patients, while the remaining ten had grade I–II neurological symptoms. It has to be emphasized that only eight of 16 patients had 3–6 months of neurological follow-up after the third course of hd-cddp: all of these eight patients showed peripheral neuropathy and their symptoms, which first recurred after the second course, progressed even after hd-cddp was stopped. All patients with grade III neurotoxicity were observed in the 6-month follow-up group (table 1). Finally, optic neuritis or reduced cone and rod function was not detected. Ototoxicity was generally evident in early symptoms but was mild in all patients.

In brief, the major toxic effect of single-agent hd-cddp would seem to be peripheral neuropathy as previously reported (1,4). The absence of relevant neurotoxicity reported by Gandara et al (2) may be related to the small patient sample size, too short an observation and/or a sex/age-dependent factor. The pattern of neurotoxicity observed in this study reflects a late-developing and progressive damage.

Moreover, the possibility of recovery from an often disabling neuropathy, as well as the importance of hd-cddp administration modalities and patient characteristics in neurotoxicity development, must be further investigated.

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ANNOUNCEMENTS

Summer Seminar in Medical Ethics and Humanities

San Francisco, California, July 28–August 8, 1987

For further information, contact: Suzanne Lomas, Division of Medical Ethics, University of California San Francisco, Rm U-575, San Francisco, CA 94143-0766 (telephone: 415-476-5808).

Third International Conference on Advances in Regional Cancer Therapy of the Association of Regional Tumor Therapy

Ulm, Federal Republic of Germany, September 9–12, 1987

For further information, contact: Conference Secretary, Mrs. G. Mutschler, Department of Oral and Maxillofacial Surgery, University of Ulm—Bundeswehrkrankenhaus, Oberer Eselsberg 40, PO Box 1220, D-7900 Ulm, Federal Republic of Germany (telephone: 07 31/176-3579).

Second International Head and Neck Oncology Research Conference

Arlington, Virginia, September 10–12, 1987

For further information, contact: Gregory T. Wolf, MD, Veterans Administration Medical Center, Clinical Studies Office (153), 2215 Fuller Rd, Ann Arbor, MI 48105 (telephone: 313-769-7100, ext 755).

Drug Delivery Systems 87

Sophia-Antipolis, France, September 14–16, 1987

For further information, contact: Mlle. Francine Canal, c/o Prof. Yves Lazorthes, Service de Neurochirurgie—CHU Rangueil, Chemin du Vallon, 31054 Toulouse Cedex, France (telephone: 33-61-53-11-33, ext 3301-3351).

The European Organization for Research and Treatment of Cancer Symposium on Recent Advances in Cancer Management

Hong Kong, September 20–23, 1987

For further information, contact: I. C. T. S., Vere and Associates, Inc, 17/F, Jubilee Commercial Bldg, 42-46 Gloucester Rd, Hong Kong (telephone: 5-279200).

Seventh Annual Bristol-Myers Symposium on Nutrition Research: The Role of Nutrition in the Origins of Disease

San Francisco, California, September 21–22, 1987

For further information, contact: Helen Shaw Miller, Program Coordinator, Office of Continuing Education, University of California, Davis, School of Medicine, 2701 Stockton Blvd, Sacramento, CA 95817 (telephone: 916-453-4390).

XIIth European Symposium on Hormones and Cell Regulation

Sainte-Odile, France, October 5–8, 1987

For further information, contact: Dr. E. Carafoli, Laboratoire de Biochimie III, Ecole Polytechnique Fédérale Suisse, Universitätsstr. 16, ETH Zentrum, CH-8092 Zurich, Switzerland.

2nd Certifying Examination of the American Board of Laser Surgery

San Francisco, California, October 10, 1987

For further information, contact: Mary Clayton, RN, President of the Board, 3201 South 16th St, Suite 2006, Milwaukee, WI 53215.

Eleventh International RES Congress and Twenty-Fourth National Meeting of the Reticuloendothelial Society

Hawaii, October 17, 21–22, 1987

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (telephone: 404-828-2601).

4th European Conference on Clinical Oncology and Cancer Nursing

Madrid, Spain, November 1–4, 1987

For further information, contact: Siasa Congresos, Paseo de la Habana, 134, 28036 Madrid, Spain (telephone: 1-457.44.95).

New Approaches in Cancer Therapy

Reisenburg, Federal Republic of Germany, November 5–7, 1987

For further information, contact: Dr. G. Schulz, Klinische Forschung, Behringwerke AG, 3550 Marburg, Federal Republic of Germany (telephone: 06421-39-22-32).

International Workshop on Monokines and Other Non-Lymphocytic Cytokines

Hilton Head Island, South Carolina, December 6–10, 1987

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (telephone: 404-828-2601).

Second International Congress on Neo-Adjuvant Chemotherapy

Paris, France, February 4–6, 1988

For further information, contact: S.O.M.P.S, Hôpital de la Salpétrière, 47 boulevard de l'Hôpital, 75651 Paris Cédex 13, France.

Third International Conference on Adjuvant Therapy of Breast Cancer

St. Gallen, Switzerland, March 3–5, 1988

For further information, contact: Secretariat Prof. H. J. Senn, MD, Department of Medicine C, Oncology Center, Kantonsspital, CH-9007 St. Gallen, Switzerland (telephone: 719-219-KSSG CH).

Bone Marrow Transplantation: Current Controversies

Tamarron, Colorado, March 6–12, 1988

For further information, contact: UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, CA 90024 (telephone: 213-206-6292).

Third International Conference on Drug Absorption

Edinburgh, Scotland, September 28–30, 1988

For further information, contact: Dr. Ian Sword, Inveresk Research International Ltd, Musselburgh EH21 7UB, Scotland (telephone: 031-665-6881).

Cancer Treatment Reports

EDITORIAL

557 Patient Accrual to Clinical Trials

ORIGINAL ARTICLES

559 Selection Factors in Clinical Trials: Results From the Community Clinical Oncology Program Physician's Patient Log

567 Synergistic Effect of Recombinant Interleukin-2 on the Inhibition of Growth of Adenocarcinoma 755 by Mitomycin

571 Influence of Daunorubicin and Cytarabine Sequencing on the Outcome of Therapy in Acute Myelogenous Leukemia: A Randomized Trial

575 Phase II Trial Evaluating Continuous Infusion of Etoposide, Cisplatin, and Hexamethylmelamine in Extensive-Disease Small Cell Carcinoma of the Lung

581 Demonstration of a Schedule-Dependent Therapeutic Synergism Utilizing the Interacting Drugs Methotrexate and Teniposide in L1210 Leukemia

593 Phase I and Pharmacokinetic Study of Menogaril Administered as a 72-Hour Continuous Iv Infusion

599 Pharmacokinetics of High-Dose Teniposide

605 Combination Chemotherapy in the Treatment of Adenocarcinoma of Unknown Primary Origin

609 Cardiotoxicity in Patients Treated With Mitoxantrone: Southwest Oncology Group Phase II Studies

615 Pilot Trial of Prolonged Continuous-Infusion 5-Fluorouracil and Weekly Cisplatin in Advanced Colorectal Cancer

619 Selective Enhancement by Menadiol of In Vitro Drug Activity in Human Lymphatic Neoplasms

627 Phase II Study of Mitoxantrone in Epithelial Ovarian Cancer

631 Activity of Bisantrene in Refractory Lymphoma

BRIEF REPORTS

635 Continuous Sc Infusion of Narcotics Using a Portable Disposable Device in Patients With Advanced Cancer

639 Salvage Chemotherapy for Non-Hodgkin's Lymphoma With a Combination of Mitoxantrone, Etoposide, Cisplatin, and Prednisolone

643 Lack of Benefit of Adjunctive Chemotherapy in Stage I Malignant Melanoma: A Southwest Oncology Group Study

645 Total-Body Irradiation and High-Dose Teniposide: A Pilot Study in Bone Marrow Transplantation for Patients With Relapsed Acute Lymphoblastic Leukemia

649 Phase II Trial of Aminothiadiazole in Previously Treated and Untreated Patients With Advanced Colorectal Carcinoma: An Illinois Cancer Council Trial

651 Hickman Catheter Clots: A Common Occurrence Despite Daily Heparin Flushing

655 Treatment of Favorable-Prognosis Nonseminomatous Testicular Germ Cell Tumors With Etoposide, Cisplatin, and Reduced Dose of Bleomycin

CLINICAL TRIALS SUMMARIES

657 Phase II Trial of Idarubicin in Patients With Pancreatic Cancer

659 Treatment of Refractory Non-Hodgkin's Lymphomas of Unfavorable Histology With Teniposide, Cytarabine, and Cisplatin

661 Phase II Trial of Etoposide in the Management of Advanced or Recurrent Mixed Mesodermal Sarcomas of the Uterus: A Gynecologic Oncology Group Study

663 Phase II Trial of Ifosfamide and Mesna in Advanced Colorectal Cancer

LETTERS

665 Synthetic Enkephalin Analog in the Treatment of Cancer Chemotherapy-Induced Vomiting

666 "Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?"

668 *Reply*

668 Drug Eruption Localized to Previously Irradiated Skin

669 High-Dose (200 mg/m^2) Cisplatin-Induced Neurotoxicity in Primary Advanced Ovarian Cancer Patients

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